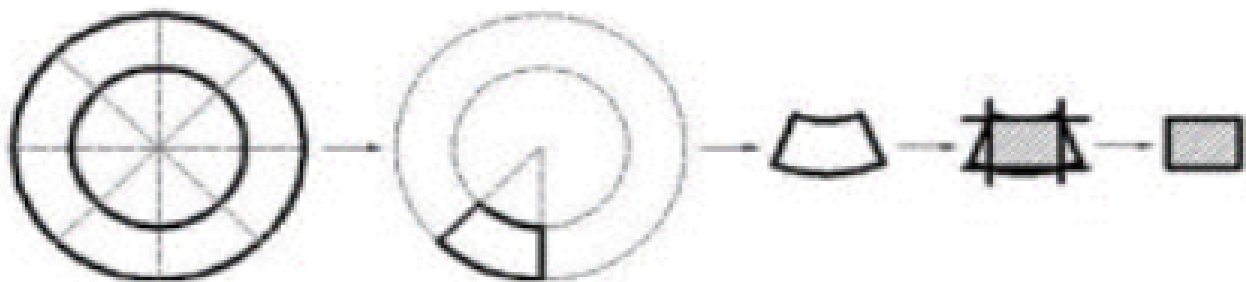


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Effect of Age and Longitudinal Culm Section of Legi Bamboo (*Gigantochloa atter* (Hassk.) Kurz) on Its Properties as Furniture and Handicraft Materials

Rini Pujiarti, Rupita Nilansari, Kasmudjo, Sigit Sunarta, Aisyah Kusumadewi, and
Brandon Aristo Verick Purba

Abstract

Legi bamboo (*Gigantochloa atter* (Hassk.) Kurz) is a common and commercially important bamboo species for furniture and craft material. The aims of this research were to investigate the optimum processing quality for furniture and craft materials of 3- and 4-year-old Legi bamboo in each culm section including processing properties and supporting information such as physical, mechanical, as well as bonding properties. The result showed that optimal processing properties of Legi bamboo had average sawing defect of 1.93% (very good); drilling defect of 26.68% (good); planing defect of 4.70% (very good); lathing defect of 7.95% (very good), and sanding defect of 2.14% (very good), considered as I – II class of processing. The results of this study indicate that the top section of 3-year-old Legi bamboo had the best physical, mechanical, and processing properties for its utilizations.

Keywords: culm height, craft, furniture, processing defect.

Introduction

Bamboo (*Gramineae*, sub-family *Bambusoideae*) is a commonly utilized tropical and sub-tropical lignocellulosic plant. It is able to live in mixed forest as a pure stand as well as cultured in plantation. Bamboo is favored for its fast growth and potential for future sustainable material, with harvest age ranged 2-5 years (Nath *et al.* 2009). In 2010, there is over 31.5 million hectares of bamboo stand or 0.8% of total area of forest in the world (Partey *et al.* 2017). *Gigantochloa atter* or Legi bamboo is a commercially important bamboo species in Indonesia. This bamboo species has been used as material for various uses such as food (shoots), handicraft, furniture, and construction material (Wahyudi 2013; Pandey *et al.* 2013). Bamboo is a versatile material that has also been processed further into various engineered products such as laminated bamboo and bamboo fiber (Imadi *et al.* 2014; Shah *et al.* 2018).

The properties of bamboo vary between age, culm height, and species. Bamboo is usually harvested at 2-5 years, but for some crafts such as bamboo cane work, utensils, and artwork require younger bamboo, usually just a few months old (Widyaningsih *et al.* 2020). Previous study by Wulandari (2019) reported that young *Dendrocalamus asper* (<2-year-old) showed weaker mechanical strength compared to mature (2-3-year-old) and old (>3-year-old) bamboo. Further, different properties and quality is also known to be varied between the culm height. In *Neosinocalamus affinis* bamboo, fiber proportion, specific gravity, vascular bundle density, as well as compression strength parallel to grain were reported to increase from bottom to top culm of the bamboo (Qi *et al.* 2014). In previous studies on other bamboo species (*Gigantochloa apus*, *G. atroviolacea*, and *D. asper*) found better

processing properties on the base section of its culm (Ahmad *et al.* 2014).

Several studies on the effect of age and culm section of bamboo has been done on several species (Ahmad *et al.* 2014; Qi *et al.* 2014, Wulandari 2019). However, there are still less information on *G. atter* (Nilansari 2014). The objective of this study was to investigate the effect of age and culm section on mechanical, physical, bonding and processing properties of *G. atter* grown in Yogyakarta, Indonesia.

Materials and Methods

Materials

Two age groups (3- and 4-year-old) Legi bamboo (*Gigantochloa atter* (Hassk.) Kurz) from Tlogoadi Village (175 m above sea level), Sleman Regency, Yogyakarta Province, Indonesia, were used as material. The bamboo culms were cut 10 cm above the ground. Each culm was cut to a length of 6 m leaving out the top part of bamboo culm (Ahmad *et al.* 2014). The bamboos were then cut at axial direction into three sections: base, middle, and top, with 2 m length in each section. The bamboos were then cut into each section of 30 cm, air dried, split, and further cut to sample sizes accordingly (Figure 1). The adhesive used was epoxy.

Physical, Mechanical, Bonding and Processing Properties

Processing properties were tested according to ASTM D-1666-64 (1985), while physical, mechanical, and bonding tests were referring to British Standard B.S.373 (1957). Moisture content (MC) and specific gravity (SG) samples were cut into 2 x 2 x thickness cm in size; width shrinkage

and volumetric shrinkage samples were cut into 2 x 4 x thickness cm. Each sample for mechanical properties testing hardness was cut into dimensions of 2 x 4 x thickness cm; compressive strength parallel to grain was tested with the dimensions of 2 x 8 x thickness cm; compressive strength perpendicular to the grain was tested with the dimensions of 2 x 6 x thickness cm. Sample for bonding quality test was performed in air dry condition and prepared with the dimension of 3.81 x 5.08 x thickness cm

by gluing together two unskinned bamboo strips. Each strip glued on one side then pressed for 72 hours. Further, whole bamboos were used for lathing test (30 cm length) while bamboo strips without the skin were used for sanding and planing tests (5 x 30 x thickness cm). Lastly, samples for sawing and drilling test have the dimensions of 5 x 30 x thickness cm (Figure 1-2). All tests were replicated three times.

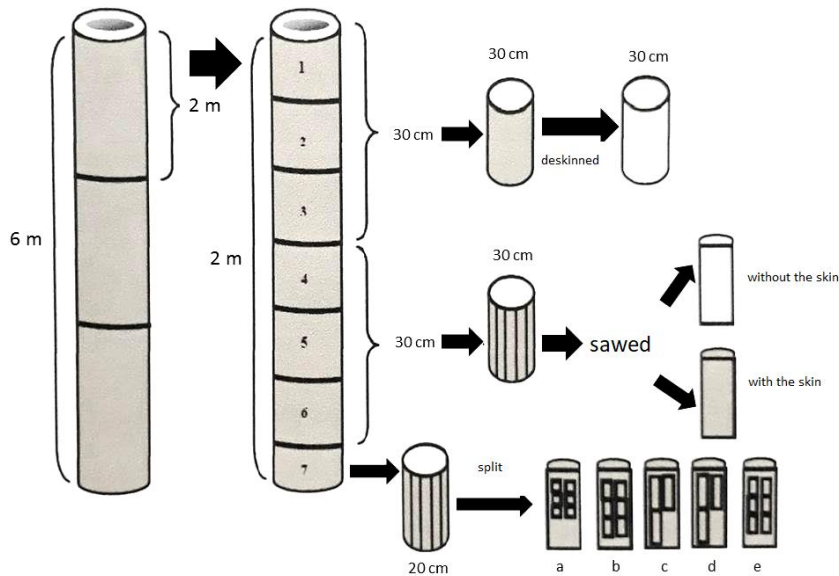


Figure 1. Sample preparation diagram

Note:

1-3 : whole bamboo without the skin for lathing test.

4-6 : bamboo strip without the skin for sanding and planing test; bamboo strip with the skin for sawing and drilling test.

- 7 :
- a. moisture content (MC) and SG samples (bamboo strip with the skin).
 - b. shrinkage and hardness test (bamboo strip with the skin).
 - c. compressive strength parallel to wood fiber (bamboo strip with the skin).
 - d. compressive strength perpendicular to the grain (bamboo strip with the skin).
 - e. bonding quality test (bamboo strip without the skin).

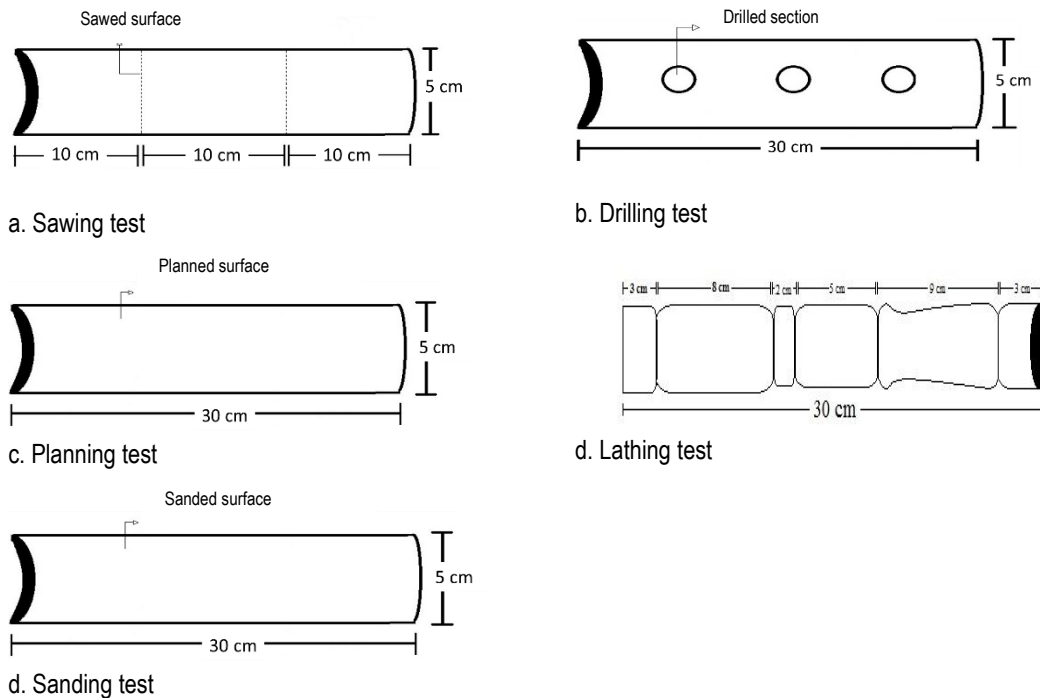


Figure 2. Processing properties test samples.

Data Analysis

Completely Randomized Design was used as experiment model with two factors: age (3-year-old and 4-year-old) and culm section (base, middle, and top). The data were analyzed with SPSS (IBM, USA) with F test in 5% and 1% confidence level and HSD (Honestly Significance Different) as post-test.

Results and Discussion

Physical Properties

The result of physical properties of Legi bamboo are showed in Table 1. Air dried moisture content varied from 12.10 - 13.40% with an average of 12.73%. This moisture content was sufficient for the equilibrium moisture content of 12 - 14% (humidity 60%) in Yogyakarta, Indonesia (Kasmudjo 2012). The moisture content was also sufficient for mechanical properties testing according to ISO 22157 (2004). The results showed that the average air dried moisture content of 3-year-old bamboo (12.29%) was lower compared to that of 4-year-old bamboo (13.18%). Further ANOVA test showed that the age factor between bamboos gave significant difference. This finding is in agreement with previous study by Falayi and Soyoye (2014) on *Phyllostachys pubescens* bamboo where 3-year-old bamboo

showed lower moisture content (37.13%) compared to 5-year-old (46.20%).

The average specific gravity value of Legi bamboo in 3-year-old (0.77) and 4-year-old (0.73) showed similar value in all culm sections. In general, top section showed the highest average specific gravity followed by middle and base section where this effect was more apparent in 4-year-old bamboo. Previous study by Qi *et al.* (2014) showed similar results where specific gravity value increase as the culm height increase in *Neosinocalamus affinis* bamboo. This effect might be showed due to the increase of cell wall thickness as the height increase in bamboo.

The average width and volumetric shrinkage on 3-year-old bamboo showed higher value compared to 4-year-old whereas 3-year-old showed width and volumetric shrinkage of 0.74% and 16.98%, respectively, and 4-year-old showed width and volumetric shrinkage of 0.59% and 16.52%, respectively (Table 1). The results showed that generally high specific gravity had high volumetric shrinkage but low width shrinkage, which especially apparent in 3-year-old bamboo. These results differ from previous studies by Qi *et al.* (2014) and Kamruzzaman *et al.* (2008), that might be caused by lower vascular bundles and other microstructure factors such as the stiffness perpendicular to the wall of Legi bamboo cells (Schulgasser and Witztum 2015).

Table 1. Physical, mechanical, and bonding properties of Legi bamboo.

No.	Sample	Physical				Mechanical			Bonding quality	
		Air dried moisture content (%)	Specific gravity	Shrinkage (%)		Hardness (kg cm ⁻²)	Compressive strength perpendicular to grain (kg cm ⁻²)	Compressive strength parallel to grain (kg cm ⁻²)	Bonding strength (kg cm ⁻²)	Bamboo failure (%)
				Width	Volumetric					
1	3-year-old, base section	12.28	0.74	0.76	14.96	96.82	166.00	494.32	53.35	21.32
2	3-year-old, middle section	12.10	0.75	0.78	15.82	112.10	126.05	570.65	33.24	47.69
3	3-year-old, top section	12.49	0.83	0.67	20.17	135.88	145.25	418.50	33.59	64.89
	Average	12.29	0.77	0.74	16.98	114.93	145.77	494.49	40.06	61.30
4	4-year-old, base section	13.40	0.73	0.50	18.55	86.62	171.90	443.80	33.49	61.98
5	4-year-old, middle section	13.18	0.75	0.58	15.47	105.31	120.91	435.66	37.84	72.95
6	4-year-old, top section	12.96	0.71	0.28	15.52	70.21	88.30	435.96	43.10	87.33
	Average	13.18	0.73	0.45	16.52	87.38	127.04	438.47	38.14	74.09
	Total average	12.73	0.75	0.59	16.75	101.16	136.40	466.48	39.10	67.69

Table 2. ANOVA result on the effect of age and culm section on the properties of Legi bamboo

Factor	df	F								
		Air dried moisture content	Specific gravity	Width shrinkage	Volumetric shrinkage	Hardness	compressive strength parallel to grain	compressive strength perpendicular to grain	Bonding strength	Bamboo failure
Age	1	9.02739 *	5.440129*	4.515755 ns	0.983556 ns	3.879871 ns	5.626462*	0.348594 ns	0.412763 ns	6.843677 *
Culm section	2	0.145461 ns	0.877023 ns	1.045822 ns	7.817549**	0.509654 ns	3.458118 ns	1.067217 ns	2.380209 ns	3.252048 ns
Interaction between factors	2	0.52097 ns	4.003236*	0.363967 ns	27.98381**	1.861655 ns	3.4864118 ns	0.37283 ns	9.238044 **	4.719 *

Note: ns = not significant; * = significant; ** = very significant

Mechanical and Bonding Properties

Mechanical and bonding properties of Legi bamboo are shown in Table 1. Hardness, perpendicular and parallel compression strength of Legi bamboo between age 3 and 4 were not significantly different (Table 2). Average value of Legi bamboo hardness was 101.16 kg cm⁻² (strength class IV); compression strength parallel to grain of 466.48 kg cm⁻² (strength class II); and compression strength perpendicular to grain of 88.30 - 171.90 kg cm⁻² (strength class V). Between culm sections, generally middle section had the highest hardness, where top section had the highest compression strength. Similar results were reported by Correal and Arbelaez (2010) on *Guadua angustifolia* bamboo, where middle and top section of the bamboo had the highest mechanical strength compared to base section. Between ages, 3-year-old bamboo had higher compression

strength perpendicular to grain compared to 4-year-old, which was also in line with the specific gravity value. This results were also in agreement with the previous study carried out by Falayi and Soyoye (2014) where MOR and MOE of the bamboo were highly related with specific gravity.

The average bonding strength of Legi bamboo in this study was 39.10 kg cm⁻² with 67.69% bamboo failure. Interaction between age and culm section factors showed significant difference with ANOVA (Table 1 and 2). Bonding strength of 4-year-old bamboo was lower than 3-year-old bamboo. Between culm section, base had the highest bonding strength, followed by top and then middle section. Further, bamboo failure of 4-year-old bamboo showed higher value compared to 3-year-old. Between culm section,

top had the highest bamboo failure, followed by base and then middle.

Processing Properties

The average processing defect value of Legi bamboo are showed in Table 3. ANOVA test showed significant different in the interaction between factor on sawing defect and significant difference in age factor in lathing defect (Table 4). Legi bamboo processing in this study showed sawing defect of 11.04% (very good); drilling 26.68% (good); planning 4.69% (very good); lathing 7.95% (very good) and sanding 2.14% (very good) and overall classified as processing class I – II. Furthermore, the most common defect on sawing and planning were found to be raised grain defect. In drilling, torn grain defect was the most common. In

lathing and sanding, fuzzy grain is the most common defect. Processing properties of Legi bamboo showed the highest defect in lathing in both ages (5.09% and 10.81% for 3-year-old and 4-year-old, respectively). In both ages, 4-year-old generally showed higher processing defects except for drilling and sanding defect which was higher in 3-year-old. This result might be caused by the thickness of cell wall which is higher in older bamboo (Schulgasser and Witzum 2015). Compared to previous research by Ahmad *et al.* (2014), Legi bamboo in this study showed better processing properties than Apus (*Gigantochloa apus*), Tutul (*Bambusa vulgaris* Schrad), and Wulung (*Gigantochloa vercillata* Munro) bamboo except for the sanding property.

Table 3. Processing properties of Legi bamboo.

No.	Factor	Defect (%)					Average (%)
		Sawing	Drilling	Planning	Lathing	Sanding	
1	3-year-old, base section	0.81	32.38	6.90	6.00	3.50	13.75
2	3-year-old, middle section	0.00	23.78	3.50	5.01	1.70	8.39
3	3-year-old, top section	3.46	24.33	1.62	4.28	1.54	7.20
	Average		26.83	4.00	5.09	2.25	9.78
4	4-year-old, base section	0.86	27.06	10.10	12.36	2.11	12.71
5	4-year-old, middle section	6.43	25.68	2.69	13.23	2.66	11.21
6	4-year-old, top section	0.00	26.85	3.36	6.84	1.32	9.75
	Average		26.53	5.39	10.81	2.03	11.22
	Total average		26.69	4.37	8.83	1.68	10.49

Table 4. ANOVA result on the effect of age and culm section on processing defects of Legi bamboo.

Factor	Df	F				
		Sawing	Drilling	Planning	Lathing	Sanding
Age	1	0.339912 ns	0.017709 ns	0.472 ns	9.764 **	0.22317 ns
Culm section	2	0.354265 ns	0.854299 ns	3.601 ns	1.709 ns	0.546441 ns
Interaction between factors	2	15.09317 **	0.614808 ns	0.339 ns	0.827 ns	0.218785 ns

Note: ns = not significant; * = significant; ** = very significant

Conclusions

Interaction of age and culm section had significantly different values in specific gravity, volumetric shrinkage, bonding strength, bamboo failure, and sawing defect values. Moisture content, specific gravity, compressive strength parallel to grain, and lathing defect values are significantly influenced by age of bamboo. Moreover, culm section had significantly different values in volumetric shrinkage only. The 3-year-old bamboo had significantly lower air-dried moisture content and higher compressive strength parallel to grain. Further, the highest specific gravity, volumetric shrinkage, and bamboo failure was showed by the top section of 3-year-old bamboo, while the highest bonding strength was showed by the base section of 3-year-old bamboo. Width shrinkage and compressive strength perpendicular to grain showed similar value in all samples. In processing properties, the highest sawing defect was showed by the middle section of 4-year-old bamboo, while lathing defect was higher in 4-year-old bamboo. Drilling,

planning, and sanding defect were similar in all ages and stem sections of the samples. Legi bamboo in this study can be classified as processing class I-II. These results indicate that 3-year-old bamboo, especially at the top section, showed the best physical, mechanical, and processing properties for its utilizations.

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The Effect of Strip Arrangement on Physical and Mechanical Properties of Petung Bamboo Laminated Board (*Dendrocalamus asper* Backer)

Febriana Tri Wulandari, Habibi, Raehanayati, and Rima Vera Ningsih

Abstract

One alternative material as a substitute for wood construction is laminated bamboo. Laminated bamboo is a product made from several bamboo strips which are glued together with the fiber direction parallel to the board with several requirements, among others, must have dimensions of length, width, and thickness that can be converted into boards or blocks. The type of bamboo that will be used in this research is petung bamboo (*Dendrocalamus asper* Backer). Petung bamboo was chosen because it has a diameter that can reach 20 cm with a wall thickness of 1-3 cm, making it suitable for use as laminated bamboo. The purpose of this study was to determine the effect of strip arrangement on the physical and mechanical properties of petung bamboo laminated boards. The method used in this study is an experimental method with a non-factorial completely randomized design experiment with two treatments and three replications. Based on the results of the study, the arrangement of the laminated board strip did not significantly affect all tests of the physical and mechanical properties of the petung bamboo laminated board. All tests of physical and mechanical properties have complied with JPIC standard No. 1152 except in the Modulus of Elasticity (MoE). The bamboo petung laminated board is classified as strength class III which can be used as a protected heavy construction material.

Keywords: physical and mechanical properties, petung bamboo, strip arrangement.

Introduction

Recently, wood as a construction material is increasingly limited. This can be seen from the data on the decreasing amount of wood products from natural forests, which were from 8.3 million m³ in 2015 to 5.7 million m³ in 2018 (Muhtariana 2013). This condition is due to the unbalanced use of wood compared to the efforts to plant new stands. Therefore, alternative materials are needed to replace wood. One alternative material as a substitute for construction wood is bamboo. One of the advantages of bamboo is that it is affordable, can grow in various fields, and grows fast. Bamboo is a fast-growing plant that can reach 15-18 cm in height in 4-6 weeks (Akinlabi *et al.* 2017). Bamboo in Indonesia varied over 143 species, of which 9 types of them are endemic on the Java island (Manik *et al.* 2017).

Bamboo is one of the non-timber forest products that need to be promoted as construction materials which is processed with high technology. The current technology that makes it possible to process bamboo into wood-like blocks is lamination technology. Laminated bamboo is a product made from several bamboo strips or bamboo peels that are glued together in parallel fiber directions (Qisheng *et al.* 2002). As a material of laminated board, bamboo has several requirements, including dimensions of length, width, and thickness that can be converted into boards or blocks. (Prabowo and Supomo 2013). The type of bamboo that will be used in this study is petung bamboo (*Dendrocalamus asper* Backer). Petung bamboo was chosen because it has a diameter that can reach 20 cm with a wall thickness of 1-3

cm, making it suitable for use as laminated bamboo (Morisco 2006).

Cahyadi *et al.* (2012) conducted a study on the effect of various addition methanol as adhesive diluent and powder weight on the physical and mechanical properties of laminated bamboo from petung bamboo. Yasin (2015) showed laminated petung bamboo with PVAc adhesive and large variations in compression pressure. Anokye (2016) conducted a study on the effect of nodes and adhesives on the mechanical properties of laminated bamboo of the *Gigantochloa scortechinii* species.

This research examines the effect of the arrangement of a bamboo strip on the strength of the laminated board, so it is necessary to do a test to see the physical and mechanical strength of the petung bamboo laminated board.

Physical and mechanical properties are indicators that can determine the quality of laminated boards. It is very important to know mechanical properties, which are properties to describe the strength of laminated wood and solid wood (Dumanauw 2001). The purpose of this study was to determine the effect of strip arrangement on the physical and mechanical properties of petung bamboo laminated board.

Materials and Methods

This research was carried out from December 2020 to June 2021. The physical test was carried out at the Forest Product Technology Laboratory, Faculty of Agriculture, Mataram University. Meanwhile, the mechanical test was carried out at the Physics Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram.

Materials used in this study was petung bamboo strip to be made of laminated bamboo with board dimensions of (2 x 8 x 38) cm³ and PVAC adhesive. The research design was a non-factorial completely randomized design (CRD) with 2

treatments with 3 replications so that there were 6 test samples, as follow:

1. Treatment of the arrangement of the joints of the strip the thick direction (S1)
2. Treatment of the arrangement of the splicing of the strip towards the width (S2).

Table 1. Research design.

Treatments	Repetition		
	U1	U2	U3
S1	SIU1	S1U2	S1U3
S2	S2U1	S2U2	S2U3

Remark:

S1 = The strip arrangement in the thick direction; S2 = The strip arrangement towards the width

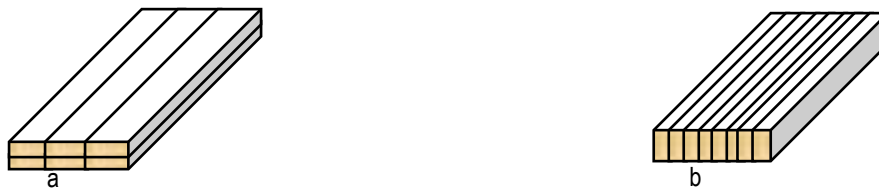


Figure 1. a) Strip arrangement the thick direction; b) Strip arrangement in the width direction.

Preparation of Raw Materials

The base of the bamboo was cut to a length of 50-80 cm to remove the crooked part of the bamboo stem. The bamboo was then cut into several pieces with a length of 40 cm, the bamboo pieces must be straight, cylindrical, and the bamboo walls are thick enough. Bamboo was air dried for 3

weeks. The splitting of the bamboo stem was carried out by paying attention to the part of the bamboo stem with a smaller diameter which was used as a reference for the cleavage trajectory. The bamboo strips used were straight bamboo on both sides of the length, then the strips were dried for one week. After that, the bamboo blades were shaved to get a flat strip surface.

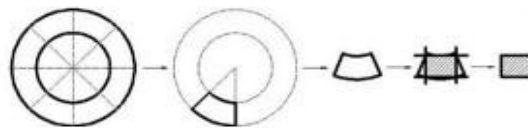


Figure 2. Bamboo strip making process (Wulandari 2012).

Assembling of Bamboo Strip

The bonding of the adhesive on the bamboo strip with glue spread 100 g/cm³ was then assembled with a predetermined arrangement of strips. Then followed by the cold press for 24 hours with a compression pressure of 20 Nm. Conditioning was carried out in a constant room for 7 days to uniform the moisture content before testing. Sanding

on both surfaces of the bamboo to even out the two surfaces.

Making Test Samples

Laminated bamboo cutting for each test sample was for moisture content and density (2 (p) x 2 (l) x 2 (t)) cm³, thickness expansion and thickness shrinkage (5 x 5 x 2) cm³, as well as MoE and MoR (30 x 2 x 2) cm³.

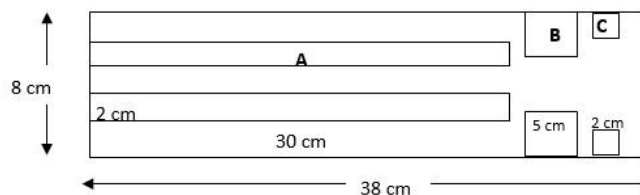


Figure 3. Test sample size.

Remark:

A: Test sample for MoE and MoR; B: Test sample for thickness swelling and shrinkage; C: Test sample for moisture content and density.

The testing of the physical and mechanical properties of laminated bamboo refers to ISO 21629-1: 2021. The data that had been obtained were then analyzed for variance ANOVA to determine whether the results were significantly different or not using the SPSS 25 prog.

Results and Discussion

The average values of physical and mechanical properties of petung bamboo laminated board are presented in Table 2 and the results of analysis of variance (ANOVA) are presented in Table 3.

Table 2. Average values of physical and mechanical properties.

Properties	Treatment		Average
	S1	S2	
Density (g/cm ³)	0.429	0.469	0.449
Moisture Content (%)	14.066	13.443	13.754
Thickness Swelling (%)	3.113	4.040	3.576
Thickness Shrinkage (%)	3.767	3.194	3.481
Modulus of Elasticity (kgf/cm ²)	14211.069	10585.980	12398.524
Modulus of Rupture (kgf/cm ²)	342.627	287.949	315.288

Remark: S1 = Arrangement of the strip towards thickness and S2 = Arrangement of strip towards width.

Table 3. Calculated F values of varian analysis.

Properties	F calculated	Sig.
Density	1.039	0.366
Moisture Content	4.114	0.112
Thickness Swelling	0.398	0.562
Thickness Shrinkage	1.522	0.285
Modulus of Elasticity	2.728	0.174
Modulus of Rupture	2.705	0.175

Density

The density of petung bamboo laminated board ranged from 0.37-0.49 g/cm³ with an average value of 0.45 g/cm³. Based on SNI 01-6240-2000 (2000) this value met the standard of laminated bamboo with a standard value of 0.40 - 0.80 g/cm³. This result was lower compared to research conducted by Priyanto and Iskandar (2019) on the use of petung bamboo laminate for building materials with an average density value of 0.63 g/cm³. The lower the density of a material, it will be followed decrease in the strength of the material (Oka 2005).

The results of the analysis of diversity analysis in Table 2 show that the arrangement of bamboo strips did not significantly affect the density of the petung bamboo laminated board which was marked with a significance value of 0.366.

Moisture Content

Moisture content can be defined as the weight of the water content in percent (Haygreen and Bowyer 2003). The value of the moisture content of the petung bamboo laminated board ranged from 13.07 - 14.49% with an average water content of 13.75%. Based on this value, the moisture content value of petung bamboo laminated board met the Japan Agricultural Standard (2003) standard with a value of 14%. This results was higher compared to the research conducted by Manik *et al.* (2017) moisture content of laminated beams with a combination of petung bamboo

and apus bamboo for wooden ship components was ranging from 11.33 - 12.40%. The factors that determine the difference in water content of a laminated product are the type of adhesive, pre-treatment, the thickness of the laminate, specific gravity of bamboo, number of layers of laminate, lamination weight, adhesive water content and the procedure used in the gluing process (hot or cold pressing) (Sulastiningsih *et al.* 2005). The value of moisture content affects the quality of the petung bamboo laminated board produced. This statement is supported by Mahdavi *et al.* (2011) that structural wood types, the mechanical strength of bamboo such as compressive strength, tensile strength, MoE, and MoR will increase as the water content decreases.

The results of Table 3 show that the arrangement of bamboo strip did not significantly affect the moisture content of the petung bamboo laminated board which was marked with a significance value of 0.11.

Thickness Swelling

Thickness swelling is the addition of thickness due to immersion in water for 24 hours (Wulandari 2012). The average value of the thickness swelling of the laminated board ranged from 2.42 - 6.94% with the average value of thickness expansion of 3.58%. The value of thickness expansion in the arrangement of the blades towards the thickness tends to be lower than the arrangement of the blades towards the width. Based on the Japan Agricultural Standard (2003) standard which requires a thickness

expansion value of 20%, the thickness development of the petung bamboo laminated board has met the standard. When compared with research conducted by Cahyadi *et al.* (2012) with a thickness development value of 5.60 - 18.30%, the thickness development value of petung bamboo was lower. Petung bamboo has a thick cell wall (0.90 microns) where the thicker the cell wall of a laminated bamboo material, the higher its ability to absorb water (Manuhuwa and Loiwatu, 2007).

The results of the varian analysis in Table 3 show that the arrangement of bamboo strips has no significant effect on the development of the thickness of the petung bamboo laminated board which is marked with a significance value of 0.562.

Thickness Shrinkage

Bamboo shrinks when dried in contrast to wood where wood shrinks from the fiber saturation point to oven dry (Manuhuwa and Loiwatu, 2007). The average value of thickness shrinkage of petung bamboo laminated board ranged from 2.73 - 4.21% with an average value of 3.48%. Thickness shrinkage value in the arrangement of the blades towards the thickness tended to be higher than towards the width. The thickness shrinkage value of petung bamboo laminated met the Japan Agricultural Standard (2003) standard with a standard value of 14%. The value of thickness shrinkage in this study was smaller than the results of Megawati *et al.* (2016) on Gerunggang wood (*Cratoxylon arborescens* Bl.) which is 6.62% and research by Hidayati *et al.* (2016) on 7.90% superior teak and 8.50% conventional teak.

The results of the analysis of varian in Table 3 show that the arrangement of bamboo strips did not significantly affect the shrinkage of the thickness of the petung bamboo laminated board which was marked with a significance value of 0.28.

Modulus of Elasticity

A high MoE value describes a material having high stiffness so that it can withstand large pressures with a small deformation value whose value is obtained by testing the static bending strength by measuring the deflection in the curved area of a material when loading occurs, the MoE value is seen if the point distance loading 1/2 the distance from the pedestal (Oka 2005). The average value of MoE in this study ranged from 9593.96 - 17952.04 kgf/cm² with an average value of 12398.524 kgf/cm². Based on Japan Plywood Inspection Corporation (2007) MoE value of laminated bamboo did not meet the standard which requires a minimum MoE value of 75000 kgf/cm². The MoE value in this study was higher than that of Arifin *et al.* (2017) which shows the average MoE value of bamboo laminated beams is 1361.63 kgf/cm². The MoE value of the arrangement of the blades towards the thickness tended to be higher than towards the width. This was in accordance with the statement of Espiloy (2000) in his research which shows

that laminated bamboo will be stronger to withstand the load when tested with the test position in the thick direction. The results of the analysis of varian in Table 3 show that the treatment has no significant effect on the Modulus of Elasticity of the petung bamboo laminated board which is marked with a significance value of 0.174.

Modulus of Rupture

Modulus of Rupture (MoR) is defined as the ability of an object to withstand the maximum load until the object breaks (Prihandini 2012). The MoR values of petung bamboo laminated board ranged from 259.574 to 399.545 kgf/cm² with an average value of 315.288 kgf/cm². The MoR value in the thick directional bar arrangement was higher than that of the wide directional bar arrangement. Based on Japan Plywood Inspection Corporation (2007) then the MoR value of the laminated bamboo petung board has met the standard which requires a minimum MoR value of 300 kgf/cm². When compared to research by Cahyadi *et al.* (2012) with a value of 210 kgf/cm², the value of petung bamboo laminated board was higher. The MoR value towards thickness tended to be higher than towards width. This was in accordance with the statement of Espiloy (2000) in his research which showed that laminated bamboo will be stronger to withstand the load when tested with the test position in the thick direction.

The results of varian analysis test in Table 3 show that the treatment has no significant effect on the MoR of the petung bamboo laminated board which was marked with a significance value of 0.175 so that further DMRT testing does not need to be carried out to determine the differences between treatments.

Conclusions

The arrangement of the strips of the petung bamboo laminated board had no significant effect on all tests of the physical and mechanical properties. All tests of physical and mechanical properties meet with Japan Plywood Inspection Corporation (2007) except for the MoE testing. Based on the strength class of the laminated board, petung bamboo laminated board was included in the strong class III which can be used as a construction material that can be used as a protected heavy construction material.

Suggestions that need to be done for further research is to pay attention to the uniformity of the bamboo to be laminated because it is seen from the unevenness of the adhesive in the adhesive smelting process will affect the strength of the laminated bamboo made and further research needs to be carried out to increase the value of the physical and mechanical properties of laminated bamboo related to the amount optimum coating weight and the use of water-resistant adhesive according to the final use of the resulting laminated bamboo.

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Bonding Characteristic of Gambir Tannin-based Adhesive on Tusam Wood (*Pinus merkusii*) in Various Ages: Effects of Gambir Leaves Condition and Extender Addition

Adi Santoso, Erlina Nurul Aini, and Dina Alva Prastiwi

Abstract

Exploration of bio-based wood adhesive as an alternative to conventional adhesives such as urea-formaldehyde (UF) and phenol-formaldehyde (PF) is an interesting topic to be investigated further due to the increase in human awareness of environmental sustainability and health. Among several bio-based adhesives, the tannin-based adhesive has considerable potential to be developed as a commercial wood adhesive. In Indonesia, one of the materials that have high potential as a raw material for making tannin-based adhesives is gambir leaves. In this study, the effects of leaf freshness, extender content, and wood age on the quality of the laminated product were studied. The results showed that all three factors significantly affect the compressive shear strength of the laminated wood. The condition of fresh leaves, 0% extender content and 27 years old wood produced a laminated product with the highest compressive shear strength i.e., 109.30 kg/cm². All laminated wood products in this study meet the compressive shear strength requirement of the Indonesia National Standard (SNI). Moreover, all laminated wood glued using tannin adhesive from fresh leaves without the addition of an extender are also able to meet the Japanese Agricultural Standard (JAS) requirement of compressive strength. The research results also show that gambir tannin adhesive has properties and bonding strength that compete with PF adhesive. From these results, gambir tannin-based adhesive demonstrates an ability to serve as an alternative to conventional adhesive commonly used in the laminated wood industry.

Keywords: Compressive shear strength, extender content, gambir tannin-based adhesive, leaf freshness, tusam wood.

Introduction

The rising awareness of both environmental sustainability and human health has triggered the efforts to use renewable and safe materials for health in various aspects of life. Regarding wood adhesive, this was done by utilizing biomaterials to produce adhesive that can serve as an alternative to the conventional adhesives generally used in the wood processing industry. Conventional adhesives such as urea-formaldehyde (UF) and phenol-formaldehyde (PF) have advantages, i.e., they are relatively inexpensive and have excellent performance as adhesives. The excellent adhesive performance of UF and PF was shown by the strong and stable characteristics of the product bonded by both adhesives (Mamza *et al.* 2014). However, UF and PF are adhesives that are derived from non-renewable materials and produce formaldehyde emissions that are harmful to human health (Raydan *et al.* 2021).

Some researchers found that renewable and non-toxic materials like starch (Gadhav *et al.* 2017), lignin (Dongre *et al.* 2015), and tannin (Dhawale *et al.* 2022) have considerable potential to be developed as a wood adhesive. Their potential as wood adhesives was related to their massive availability in nature, which led to their economical price compared to conventional adhesives. Natural based adhesive also has a bonding ability that can be on par with conventional adhesives (Müller *et al.* 2007; Gonultas 2018). Among the natural material-based adhesives, tannin adhesive is quite an attractive option for further

development, especially since the development of tannin adhesive has reached the commercialization stage (Pizzi 2016). Tannin comprises several complex organic compounds and has high reactivity toward aldehydes and other reagents, especially in condensed forms (Pizzi, 2008; Zhang *et al.* 2022). The high reactivity was attributed to high phenolic compound content in tannin, which makes tannin can be used as an adhesive or as a substitute for phenols in adhesives (Pizzi 2006; Zhou and Du 2019). According to Santoso *et al.* (2012), tannin-formaldehyde adhesive emits significantly less formaldehyde than UF and PF adhesives, which becomes an additional advantage of tannin-based adhesive.

As a country with high biodiversity, Indonesia has many sources of tannins that can be used to produce adhesives. Tannin from gambir (*Uncaria gambir* (Hunter) Roxb.) leaf is one of the many sources of tannins available in Indonesia. Gambir plants in Indonesia are mainly present on Sumatra Island, especially in West Sumatra Province, where about 80% of total national gambir production comes from this province (Isnawati *et al.* 2012). In 2020, BPS (2021) recorded that the gambir plantation area in West Sumatra was about 28.016 ha. The gambir plantation area is predicted to increase further in West Sumatra and other potential locations in Indonesia. It is due to the Ministry of Agriculture's plan to increase Indonesia's gambir production up to 500% between 2020-2024 (Kementan 2020).

Adhesive from gambir extract has also been shown to have good bonding performance that can meet quality

standards from Indonesia and Japan. Malrianti *et al.* (2018) did research to produce a cold-press adhesive from gambir-tannin extract with an addition of 10% hexamethylenetetramine. That research found that laminated wood bonded using gambir-tannin cold setting adhesive with a glue spread rate of 200 g/m² and pressing time of 24 h has compressive shear strength between 11.5 to 21.1 kg/cm². Other research about gambir-tannin as an adhesive was also done by Sucipto *et al.* (2020). The application of gambir-tannin-sucrose as a hot-press adhesive in the production of bamboo particleboard resulted in excellent internal bonding strength of the board that reached 0.89 MPa (Sucipto *et al.* 2020). The particleboard manufacturing condition used was gambir-tannin/sucrose composition of 25/75wt% and press temperature of 200°C for 10 minutes. The effective polymerization reaction via the methylene bridge occurred between tannin-hexamethylenetetramine and tannin-hydroxy-methyl-furfural (5-HMF) derived from sucrose contributed to the excellent bonding strength of these gambir adhesives.

The glued product quality is broadly influenced by the adhesive material, adherend, the gluing process, and the product use conditions (Sucipto and Ruhendi 2012). Regarding the adhesive factor, an adhesive component consisting of the main component and additional components, such as an extender, plays a crucial role in determining the quality of the adhesive and the bonded product. In bioadhesive that used biomass extract as the main component, the condition of the extracted raw material, like its freshness, will affect the quality of the produced extract (Abdul Razak *et al.* 2014). While in adherend factors such as wood, the natural properties of wood will also have a major influence on the bonding quality and the overall glued product properties. According to Basri *et al.* (2012), the natural properties of wood can be influenced by its age. The difference in natural wood properties may affect its adhesion properties. Therefore, this study investigated the effect of the freshness condition of gambir leaves as a source of tannins on the quality of the gambir tannin-based adhesive. This study also examines the effect of leaf freshness, extender content, and wood age on the laminated product's compressive shear strength. This research was expected to provide information for the benefit of further development of gambir tannin-based wood adhesive. Tusam wood (*Pinus merkusii* Jung et de Vriese) is used as the substrate because it is one of the most accessible types of wood to find in Indonesia. It also has been widely used in wood composite products manufacture.

Materials and Methods

The materials used in this study were gambir (*Uncaria gambir* (Hunter) Roxb.) leaves in fresh and dry conditions, tusam wood (*Pinus merkusii* Jung et de Vriese), distilled water, technical grade formaldehyde solution (HCOH, 37%), and technical grade sodium hydroxide solution (NaOH, 40%). An industrial wheat flour also used in this research,

as an extender. Fresh gambir leaves were obtained by taking them directly from the tree during the gambir harvest period, while dry gambir leaves were taken from leaves that fell to the ground. The tusam wood used in this study was in age 17, 21, 23, 27, and 28 years old. The equipment needed in this research was a measuring cup, a measuring flask, a burette, a soxhlet apparatus, a porcelain dish, a water bath (Laboratory Water Bath WNB 29 Ring, Memmert, Germany), a cold press (Local Build, Indonesia), an oven (Universal Drying Oven UN 500, Memmert, Germany), a moisturemeter (Mini-Ligno E/D Moisturemeter, Lignomat, USA) a pH meter (pHTestr 30, Eutech Instruments, Singapore), a universal testing machine (LLYOYD Instrument EZ 20 Material Testing Machine, Ametek Inc., USA), a pycnometer (PICNO-25 ml, IWAKI, Indonesia), a viscometer (Viscotester VT-04E, Rion, Japan), an extractor (Local Build, Indonesia), and a reactor (Local Build, Indonesia).

Analysis of Gambir Leaves Characteristics

The analysis of gambir leaf characteristics was carried out in this study by water content and tannin content evaluation. Both analyses were carried out with three (3) replications per type of leaf condition. The water content of gambir leaves was determined using the oven drying method with a temperature of ±105°C following the AOAC method (2005). Meanwhile, the tannin content determination was completed using the permanganometry method (Depkes RI 1995). The analysis was done by dissolving gambir leaves into distilled water using a heating process. A number of the filtered solutions were mixed with the indigo carmine indicator. Then the solution mixture was titrated using 0.1 N Potassium Permanganate (KMNO₄). Titration was finished until the solution color changed from blue to green and finally golden yellow. The volume of KMNO₄ added was recorded. Blank titration was also carried out using KMNO₄ solution to calculate tannin content. The amount of tannin content contained in gambir leaves was calculated using the equation from Atanassova and Christova (2009) as stated in Formula 1.

$$KT = ((V1 - V2) \times 0,004157 \times fp) / B \times 100 \dots \dots \dots (\text{Formula 1})$$

with:

- KT = Tannin content (%)
- V1 = KMNO₄ volume for tannin titration (ml)
- V2 = KMNO₄ volume for blank titration (ml)
- fp = dilution factor
- B = gambir leaf sample weight (g)
- 0,004157 is the equivalence number of tannins to 1 ml KMNO₄ 0.1 N

Tannin Extraction from Gambir Leaves

The initial stage of the gambir leaves tannin extraction process is done by soaking the gambir leaves in an

extractor using distilled water heated to a temperature of 70-80°C. This gambir extraction method followed the extraction method done in Santoso Abdurrahman (2016) and Hendrik *et al.* (2019). Leaf composition : distilled water used was 1 : 3. After that, the mixture of gambir leaves and distilled water was heated for about 1 hour at a temperature of $\pm 90^\circ\text{C}$. The gambir leaves and distilled water mixture were stirred every 15 minutes during the heating or boiling process. After the heating process was complete, the mixture of gambir leaves and distilled water was cooled and then filtered using general filter paper. After that, the gambir leaf residue from the first extraction was extracted again in the same way for two (2) times more. The total extraction cycles performed were three (3) extraction cycles. The obtained liquid tannin was then stored in a closed container.

Preparation of Gambir-Tannin-Formaldehyde Adhesive

The manufacture of tannin-formaldehyde (TF) adhesive was carried out in several stages. The first step was to mix the liquid gambir-tannin extract with 37% formaldehyde solution and 40% NaOH in the reactor. The ratio of tannin extract : 37% formaldehyde : 40% NaOH used was 30 : 6 : 1. The pH of the solution was adjusted to 11 using NaOH (40%). The mixture was then heated to 90°C for 3 hours. During this, it was hypothesized that a polymerization reaction between tannin and formaldehyde via methylene bridge linkage would occur, resulting in a tannin-formaldehyde polymer (Zhou and Du 2019). The solution was agitated at a slow speed, and the pH was controlled at 11 every 1 hour using NaOH. After heating at 90°C for 3 hours, the reactor temperature was increased to 100°C, and let the reactor was run for ± 1 hour to reduce further the water contained in the solution. If the water content was further reduced, it was expected that the TF adhesive's viscosity would increase and could meet the viscosity standard of PF adhesive. The reactor was then turned off. The solution in the reactor was conditioned until it reached room temperature and a pH of 11. Next, the TF solution was stored in a closed container. Prior to applying TF adhesive, wheat flour extender was added at 0%, 5%, 10%, 15%, and 20% levels based on the weight of the adhesive. The mixture was then stirred manually until homogenous.

Analysis of Gambir-Tannin-Formaldehyde Adhesive characteristics

The adhesive characteristics were analyzed, including visual tests, gelation time, solid resin content, viscosity, acidity (pH), and specific gravity. Visual testing was done by observing the color and the presence of impurities in the adhesive. Gelation time evaluation was completed by boiling the TF adhesive at $\pm 100^\circ\text{C}$, which had been put inside a test tube before until it showed a limited flowing characteristic when the test tube containing TF adhesive was tilted. The assessment of solid content was carried out using the gravimetric method. TF adhesive sample was

dried in the oven at $103\pm 2^\circ\text{C}$ until it reached constant weight. Adhesive weight before and after the drying process was used to calculate its solid content. Viscosity, acidity, and specific gravity properties were evaluated at room temperature conditions using a viscometer, pH meter, and pycnometer, respectively. All adhesive analyses were carried out based on the standard SNI 06-4567-1998 (SNI 1998) with three (3) replications for each treatment.

Laminated Wood Manufacture

The manufacture of laminated products from tusam wood started with wood preparation. At this stage, the logs were cut into 20 cm x 6 cm x 1 cm. After being cut, the wooden slats were then dried using an oven at a temperature of $\pm 50^\circ\text{C}$ to reach a moisture content of $\pm 12\%$. The next stage was the gluing process using TF adhesive. The extender level used is 0%, 5%, 10% 15%, and 20% based on adhesive weight. The adhesive was spread evenly onto the surface of the wooden slats. The glue spread rate used is 170 g/m². After the adhesive was spread evenly, the wooden slats were cold-pressed at room temperature for 24 hours. The number of replications used in the manufacture of laminated wood was three (3) replications per treatment combination.

Evaluation of Laminated Wood Compressive Shear Strength

The laminated wood was left for ± 7 days for conditioning before the product property was evaluated. The evaluation of laminate product property was only in the form of compressive shear or block shear strength testing. The shear strength analysis of the sample in this study was carried out based on the JPIC No. standard. 243 (2003) using a Universal Testing Machine.

Analysis of Tusam Wood Extractive Content

Analysis of wood extractive content completed in this research included the determination of cold-water-soluble extractive, hot-water soluble extractive, and alcohol-benzene soluble extractive contents. Analysis of cold-water and hot-water soluble extractive content was done by referring to the method in SNI 01-1305-1989 (SNI 1989a). Meanwhile, the alcohol-benzene soluble extractive content was tested based on SNI 14-1032-1989 (SNI 1989b). All measurements of extractive content were repeated 3 times for each wood age. For cold-water extractive content analysis, it was done using several steps. Firstly, the wood sample was immersed in room temperature water for 48 h, then dried the wood sample using the oven for 4 hours at $103\pm 2^\circ\text{C}$. The drying process was done until the wood sample weight was constant. Hot-water extractive content analysis was conducted by putting an Erlenmeyer flask with a mixture of wood samples and distilled water into a water bath containing boiling water for 3 hours. The hot-water extracted wood sample was then dried using the same method as drying the wood sample after cold-water

extraction. The cold-water and hot-water extractive content were calculated using their weight before and after the extraction.

Alcohol-benzene extractive content evaluation was completed by putting the wood sample in filtering paper (Whatman No. 1 filter paper). The filter paper was put into a petri dish and then placed inside a soxhlet apparatus. A 1:2 alcohol-benzene solution was added inside the apparatus. The extraction process was conducted using a water bath for 6 hours. After that, the filtering paper containing samples was removed from the apparatus. The produced extraction solution was steamed until nearly dry. The residual evaporation was then heated in the oven at $103\pm 2^\circ\text{C}$ for 3 hours or until it reached constant weight. The alcohol-benzene extractive content was estimated using wood sample dry weight before extraction and residual evaporation dry weight.

Statistical Analysis

All data were recorded to calculate its average value and standard deviation using the Microsoft Excel program (Microsoft Office Excel 2013, Microsoft, USA). For the extractive content analysis data and compressive shear strength data, the 3-way analysis of variance using the IBM SPSS program (IBM SPSS Statistics Base 22.0 for Windows, IBM, USA) was also carried out, continued by Tukey's HSD test to understand subgroup differences among the different experimental and control groups (Lee and Lee 2018). Tukey's HSD test was chosen for this research data analysis because it tests all pairwise differences. It is also simple to compute and can reduce the probability of making a Type I error (McHugh 2011). Type I error in statistics means rejecting the null hypothesis when it is actually true; hence committing this type of error is considered riskier compared to other errors (Kim 2015).

Table 1. Water content and tannin content of gambir leaves

Leaf condition	Water content (%)	Tannin content (%)
Fresh	63.67±0.86	18.16±0.88
Dry	17.16±0.41	15.91±0.94

Table 2. Characteristics of tannin formaldehyde adhesive from gambir leaves

Adhesive Characteristics	Adhesive type		
	TGFB	TGFK	PF standard
Color	Brown-black	Brown-black	Brown-black
Odor	Phenolic typical	Phenolic typical	Phenolic typical
Gelation time (minute)	94	87	30-60
Solid resin content (%)	26.01	28.95	41-43
Viscosity (25±1°C), (Poise)	2.85	2.95	1.5-3
Acidity / pH	10.35	10.32	10-13.6
Specific gravity	1.08	1.06	1.18-1.20

Note : TGFB = Tannin formaldehyde adhesive from fresh gambir leaf extract
 TGFK = Tannin formaldehyde adhesive from dry gambir leaf extract
 PF = Phenol formaldehyde
 PF standard refers to the properties of PF adhesive stated in SNI 06-4567-1998 (SNI 1998)
 Gelation time was measured at a temperature of 100°C

Results and Discussion

Gambir Leaf Characteristics

As the main component of tannin-based adhesives, the quality of tannins was quite an important parameter related to their quality as a wood adhesive. The tannin quality, such as tannin content, can be affected by the freshness condition of the tannin source material (Kanto *et al.* 2008). The analysis results of the gambir leaves used as tannin sources in the manufacture of tannin-based adhesives in this study are listed in Table 1.

From the results in Table 1, it can be seen that the tannin yield of fresh gambir leaves was about 3% higher than dry gambir leaves. Similar results were obtained in Stewart *et al.* (2000) study. The extraction from *Calliandra calothyrsus* leaves without drying treatment produced higher tannin content than leaves with drying treatment. This could

be explained by the occurrence of thermal degradation of phenolic compounds, including tannins, due to the heat present during the drying process (Jeong *et al.* 2004). The degradation of phenolic components does not only occur at high drying temperatures but also occurs at air drying temperatures (Miranda *et al.* 2010). In addition, Martin-Cabrejas *et al.* (2009) theorized that the decrease in tannin content in dried gambir leaves was due to changes in the polyphenol structure during the drying process. The change in tannin structure makes tannin extraction more difficult. Based on the tannin content in the production of gambir tannin-based adhesive, it would be better to use fresh gambir leaves as a tannin source. However, more caution in storing fresh gambir leaves was needed to avoid leaf decomposition. Fresh gambir leaves have a moisture content of 64%, which is much higher than dry gambir leaves, i.e., 17%. Leaf water content in the range of 60% is

the optimal water content value to support the leaf decomposition process (Kurnia *et al.* 2017). The decomposition process that occurs in biomaterials will cause a change in their chemical content, such as the reduction of polyphenols content, including tannins (Zhang *et al.* 2013). Therefore, in storing fresh gambir leaves for tannin production, extra care needs to be taken so that the quantity and quality of the produced tannins do not decrease. Fresh gambir leaves, in particular, should not be stored in direct contact with soil to minimize decomposition.

Gambir Tannin Formaldehyde Adhesive Characteristics

The result of the Gambir-tannin-formaldehyde (TF) adhesive characteristics evaluation is in Table 2. The results showed that all adhesive characteristics of TGFB and TGFK adhesives except solid resin content, gelation time, and specific gravity met the standard for PF adhesive according to SNI 06 4567 (1998). The viscosity of the adhesive will affect its flow and even the distribution level of the adhesive on the bonded material surface (Karliati *et al.* 2014). Meanwhile, the adhesive pH in this study was deliberately conditioned under alkaline pH conditions. Alkaline pH condition was aimed to slow down the adhesive polymerization reaction during storage, hence making the adhesive remain stable in liquid form (Santoso 2003). Besides maintaining the stability of the liquid adhesive form, an alkaline pH of adhesive also resulted in a more durable adhesion or bonding than the adhesive with an acid pH. (Wangaard *et al.* 1946; Blomquist *et al.* 1949).

The specific gravity of a liquid product, like adhesive, can reflect its relative weight and molecular weight (Stauffer *et al.* 2008). In this research, gambir TF adhesive's specific gravity was slightly lower than PF adhesive. This could be indicated that gambir TF adhesive polymer molecular weight was also lower than PF adhesive. Molecular weight and the degree of polymerization have a close relation. The high degree of polymerization tends to result in a high polymer molecular weight (Balani *et al.* 2014; Saminathan *et al.* 2014). Makkar *et al.* (1990) and Zanetti *et al.* (2014) stated that high tannin content might lead to high polymerization. As mentioned previously, gambir tannin content obtained in this research was considered low (16-18%). This could lead to the low degree polymerization and specific gravity of gambir TF adhesive compared to PF adhesive.

The gelation time test showed that the gelation time of TGFB and TGFK adhesives were about 56% and 45% longer than the upper limit value at the standard gelation time of PF (60 minutes). Gelation time is the time required for an adhesive to form a hard gel or have a very high viscosity, which makes it can not be used anymore (Aulitata *et al.* 2021). Dunky and Pizzi (2002) stated that the reactivity of an adhesive could be seen from its gelation time. The low reactivity of an adhesive might lead to a long gelation time. It can be concluded that the gambir TF adhesive in this research has a longer gelation time due to its lower reactivity compared to the PF adhesive. The low tannin

content in gambir extract prompted this lower reactivity of gambir TF adhesive. Hafiz *et al.* (2020) found that with the increase of tannin addition on tannin-phenol-formaldehyde adhesive, its gelation time was increased too.

Besides its content, the gambir tannin's low reactivity could have a role too in the long gelation time of the gambir-TF adhesive. In addition to its composition, the low reactivity of gambir tannin may contribute to the long gelation time of the gambir-TF adhesive. Khiari *et al.* (2017) conducted research using the lyophilization technique to increase tannin reactivity. The degree of reactivity of lyophilized tannin was found to be even greater than that of condensed tannin, resulting in a shorter gelation time for lyophilized-tannin adhesive compared to condensed tannin-adhesive. Kassim *et al.* (2011) discovered that the stiansy number of gambir-tannin extracted with hot water was 12 to 28% lower than the stiansy number of ethanol-extracted tannin and ethyl ethylene-extracted tannin. The stiansy number could be used to predict the amount of condensed tannin, a highly reactive tannin component, in an extract (Garro Gavlez *et al.* 1996). Tannins with a lower stiansy value tended to be less reactive. The hot-water extraction technique utilized in this study may not be ideal for extracting condensed tannin. Consequently, the reactivity of gambir tannin was likely low; consequently, the gelation time of TF adhesive was prolonged.

In contrast to the gelation time value, the solid resin content value of TGFB and TGFK adhesives was 14-17% lower than the solid resin content in the PF standard (43%). Solid resin content describes the amount of resin formed as a result of the polymerization of TF adhesive. One of the reasons that caused the low value of gambir TF adhesive's solid content was the low value of tannin content. The low tannin extract was predicted to make the reaction between tannin and formaldehyde to form TF or resin polymer less effective, causing the lesser solid content. Besides that, the low solid content was probably also caused by the high content of non-tannin components in this research's gambir extract, which was about 80%. Pizzi and Scharfetter (1978) explained that non-tannin components would reduce the actual solid content of TF adhesive because they did not take part in the reaction to form resin. Therefore, they could hamper the final properties of the obtained resin.

The solid resin content of the adhesive can affect other properties of the adhesive, such as bonding ability (Xing *et al.* 2006; Chen *et al.* 2015). Bonding ability has a linear relationship with solid resin content. The higher the solid resin content, the higher the number of molecules in the polymer expected to play a role in the reaction between adhesive and adherend. This opinion was also conveyed by Sucipto *et al.* (2020), who stated that the bonding power of an adhesive could be represented by its solid resin content. Adhesive with high solid resin content typically also has high bonding ability due to the high content of the main active binder in the adhesive able to react with wood. With a lower solid resin content, it became a concern that gambir TF

adhesive in this study will have lower bonding strength than the standard PF adhesive.

Based on the result of adhesive characteristics evaluation, it can be seen that the reactivity of gambir-TF adhesive had not reached the optimum level yet. According to Griyanitasari *et al.* (2019), DSC analysis results of the gambir extract showed that the melting point of tannin gambir occurred at $\pm 200^{\circ}\text{C}$. This melting point could represent the optimum temperature for gambir-tannin to react with another compound. The research done by Sucipto *et al.* (2020) also supports this statement. In Sucipto

et al. (2020) research, the insoluble matter of gambir adhesive reached the highest value when the adhesive was heated at a temperature of around 200°C . The measurement of insoluble matter content could indicate the degree of polymerization of the adhesive (Umamura *et al.* 2017). In order to produce TF adhesive with optimal properties, it may be necessary to use a higher temperature or to add other compounds that can reduce the optimal tannin-gambir reaction temperature during the production of gambir TF adhesive.

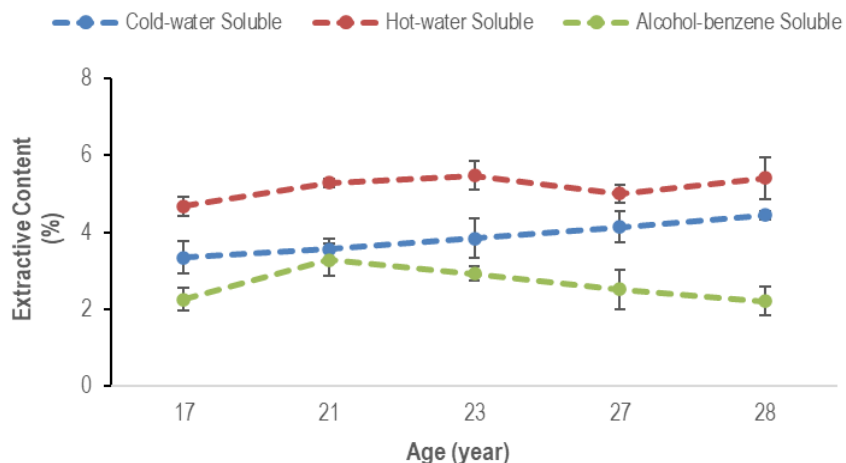


Figure 1. Tusam wood extractive content at various ages

Tusam Wood Extractive Content

Wood is a natural material composed of several chemical components, of which one of them is extractive. Wood extractive substances consist of organic and inorganic substances that are included as non-structural components of wood that can be extracted from wood using various solvents such as water, ethanol, acetone, hexane, toluene, and other kinds of solvents (Pattiya 2018). Based on the substance nature, wood extractives can be classified into 4 groups: the volatile extractive group, the soluble extractive group in neutral solvents, the cold-water-soluble extractive group, and the hot-water soluble extractive group. Wood cold-water-soluble extractive generally consists of tannins, gums, simple sugars, and salts or minerals. In contrast, the substances present in hot-water soluble extractives are tannins, gums, simple sugars, salts or minerals, and several phenolic components (Meena and Nimkar 2016). Meanwhile, neutral solvent-soluble extractives such as alcohol-benzene are relatively composed of oleoresin, fat, and wax groups (Nimkar *et al.* 2010). Wood extractives often affect the use and processing of wood (Yoshimoto 1989). Therefore, it is essential to know what types of extractives are contained in the wood to determine the appropriate wood processing technique. In this study, the extractive content of tusam wood at different ages was analyzed. The extractive content analysis was

done by determining the cold-water-soluble, hot-water soluble, and alcohol-benzene (1:2) soluble extractive content in tusam wood at different ages.

The content of cold-water extractive, hot-water extractive, and alcohol-benzene extractive was in the range of 3.35-4.45%, 4.68-5.48%, and 2.22-3.28%. The concentration of polar extractive tends to increase as a tree ages, whereas the concentration of non-polar extractive decreases (Rochman *et al.* 2008; Lachowicz *et al.* 2019). Polar extractives are typically contained in cold-water and hot-water extractives. In contrast, non-polar extractives are generally included in neutral solvent-soluble extractives such as alcohol-benzene. The result of cold-water-soluble extractive content and benzene alcohol soluble extractive content measurement in this study was similar to Rachman *et al.* (2008) and Lachowicz *et al.* (2019). However, it was found that hot-water soluble extractive content at all wood ages found in this research was relatively the same. The hot-water soluble content in this research is different from the result obtained by Rachman *et al.* (2008) and Lachowicz *et al.* (2019). The difference can occur because the extractive content in wood is affected by many other factors such as species, growing location, and even the wood storage method and duration (Silvério *et al.*, 2008; Chauhan *et al.* 2020).

Table 3. Analysis of variance of the effect of tree age on extractive content.

Factor	Cold-Water Soluble Extractive Content	Hot-Water Soluble Extractive Content	Alcohol-benzene Extractive Content
Significance (p-value)	2.83 x 10 ⁻² *	7.47 x 10 ⁻² ns	2.58 x 10 ⁻² *

Note: * = significant at 95% test level or p < 0.05, ns = not significant at 95% test level or p > 0.05

Shear Strength of Tusam Wood Laminate

Compressive shear strength can represent the bonding strength of an adhesive or the bonding quality presented in the composite board. Overall, the adhesive bonding strength can drastically affect the resulting laminate product's quality, such as its stability and mechanical strength. Figure 2 and Figure 3 show the value of the compressive shear strength of tusam wood laminated boards produced in this study. On the laminated board with TGFB adhesive, the shear strength values of the dry blocks respectively ranged from 10.06 – 74.17 kg/cm², 10.41 – 82.04 kg/cm², 9.44 – 87.61 kg/cm², 8.46 – 109.30 kg/cm² and 8.78 – 79.64 kg/cm² at wood age of 17 years, 21 years, 23 years, 27 years and 28 years. Meanwhile, for TGFK adhesives, laminated wood boards made from 17 years, 21 years, 23 years, 27 years, and 28 years old wood have a

shear strength value of 12.14 – 38.29 kg/cm², 13.94 – 49.29 kg/cm², 18.57 – 34.27 kg/cm², 13.94 – 65.85 kg/cm² and 12.83 – 37.83 kg/cm².

All laminated wood in this study met the criteria for compressive shear strength values in dry conditions required by SNI (1998), i.e., 10 kg/cm². In this study, some tusam laminated woods also met the standard of compressive shear strength required by JAS (1998). According to the JAS (1998) standard, the compressive shear strength of laminated wood is ideally around 54 – 96 kg/cm². Laminate wood that successfully met the JAS standard was all laminated wood with TGFB adhesive at 0% extender content, 17 years old laminated wood with TGFB adhesive and 5% extender, and laminated wood made of 27 years old wood with TGFK adhesive and 5% extender.

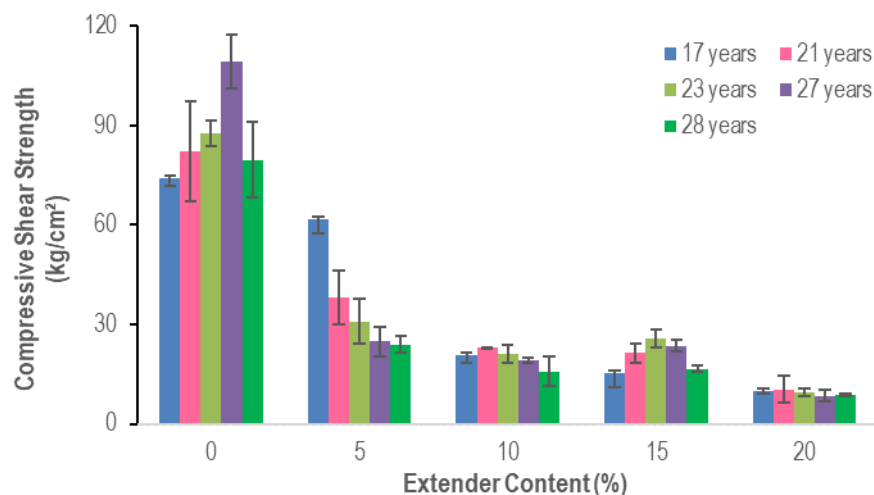


Figure 2. The compressive shear strength of tusam laminated wood in various wood ages bonded using TGFB adhesive with different extender content

The value of gambir tannin adhesive strength obtained in this study was 6-418% higher than gambir tannin adhesive produced by Malrianti *et al.* (2018). In Malrianti *et al.* (2018), the compressive shear strength values of Gambir tannin adhesive laminated board were 11.5 – 21.1 kg/cm². The difference was probably caused by the adhesive manufacturing method used. The compressive shear strength data show that the Gambir tannin adhesive synthesis method employed in this research produced superior results compared to the previous study. The good adhesion strength shown by gambir-TF glue adhesive could be linked to the polymerization reaction between tannin and formaldehyde via the methylene bridge, as stated in prior

TF adhesive study. The formation of methylene bridge on TF adhesive can be proved by the formation of a new peak around 2800-2900 cm⁻¹ at FTIR spectra of TF adhesive (Rachmawati *et al.* 2018). The average compressive shear strength value in this study was also comparable to the compressive shear strength value on laminated pinewood board bonded with PF adhesive in Júnior (2010) study, which had a value of 37 kg/cm². Previous concern about the lower adhesive power of gambir tannins from PF adhesives due to their solid resin content, which is about 40% lower than PF, did not occur. Some laminated pine wood in this study even obtained a shear strength value of 3-195% greater than PF adhesive laminated wood. In this study, it can be concluded that

the solid resin content does not necessarily represent the adhesive strength of an adhesive. A similar result also happens in the research done by Salleh *et al.* (2015). It was found that although the solid content of oil palm starch adhesive (27%) was lower than urea-formaldehyde

(UF) adhesive (51%), both adhesives were able to produce rubberwood particleboard with relatively same bonding strength, i.e., ± 0.4 MPa at the same adhesive content (15%) and press temperature (165°C) (Salleh *et al.* 2015).

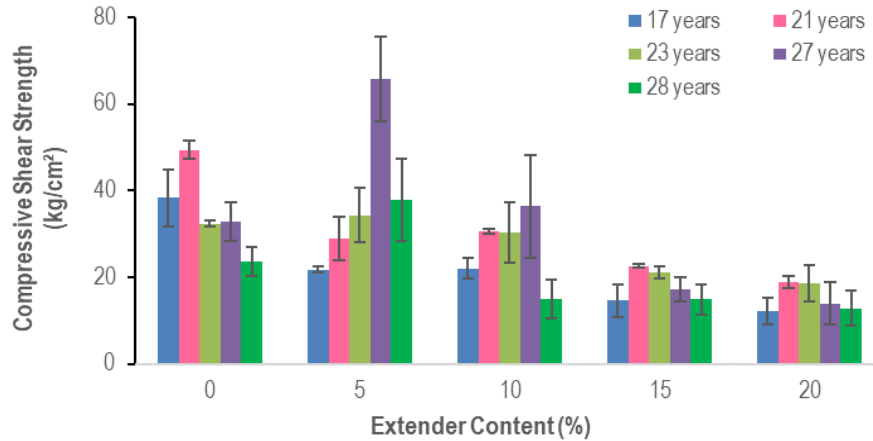


Figure 3. The compressive shear strength of tusam laminated wood in various wood ages bonded using TFGK adhesive with different extender content

Table 4. Analysis of variance in compressive shear strength of laminated wood

	Factor	Compressive Shear Strength
Significance (p-value)	Leaf condition	1.43×10^{-7} *
	Wood age	1.47×10^{-4} *
	Extender content	1.83×10^{-42} *
	Leaf condition x Wood age	5.83×10^{-3} *
	Leaf condition x Extender content	7.60×10^{-26} *
	Wood age x Extender content	0.11 ^{ns}
	Leaf condition x wood age x Extender content	1.55×10^{-8} *
	Note: * = significant at 95% test level or $p < 0.05$, ns = not significant at 95% test level or $p > 0.05$	

Compared with the adhesive strength of Merbau tannin adhesive in Santoso *et al.* (2016) research, the bonding strength of the gambir tannin adhesive in this study was able to produce 38-145% higher bonding strength. Santoso *et al.* (2016) reported that laminated bamboo with tannin adhesive from Merbau bark extract with 10% resorcinol produced the best compressive shear strength, which was around 44.7 kg/cm². The adhesive strength of an adhesive itself can be described by the value of the shear strength of the block or the compressive shear strength. According to Réh *et al.* (2021), along with increasing levels of tannins in the adhesive, the strength of the adhesive increases too. The result of gambir tannin adhesive strength in this study which was higher than that of the Merbau bark tannin adhesive in the study of Santoso *et al.* (2016), is an interesting finding. Although the tannin content in gambir leaves in this study was 11-13% lower than Merbau bark tannin content (29%) in the study of Malik *et al.* (2016), they were able to produce better adhesive strength.

The analysis of compressive shear strength results in Table 4 shows that all research factors and interactions between factors except the interaction between wood age

and extender content significantly affect the compressive shear strength of laminated wood. From the result of the compressive shear strength test, it is known that gambir leaves with the fresh condition can produce formaldehyde tannin adhesive, which has better bonding strength compared to adhesive from dry leaves. It is presumably due to higher tannin content in fresh leaf extract; hence it can produce an adhesive with better bonding strength.

Meanwhile, the HSD analysis on the age factor showed that the compressive shear strength of the laminated wood at the age of 17, 21, 23, and 27 years was relatively the same. The value of the compressive shear strength of wood at 21, 23, and 27 years was significantly different from that of 28 years old wood. There is a trend of increasing compressive shear strength until a certain age point (from 17 years old to 27 years old), but after that, there is a significant decrease in compressive shear strength (28 years old wood). This might be due to the differences in the basic properties of wood between different ages of wood, for example, the extractive substances. Nazri *et al.* (2009) stated that wood extractive constituents could vary even though the species of wood are the same. One of the

factors that influenced it was the difference in the tree's age. If this founding was associated with the results of the extractive content of the wood in this study, it is suspected the extractive compounds found in the cold-water-soluble extractive and benzene alcohol soluble extractive were more significantly affected the bonding quality of tusam wood laminated board.

One interesting thing presented in this research is the effect of alcohol-benzene extractive on laminated tusam board compressive shear strength. In this study, the highest compressive shear strength was produced by wood with the smallest alcohol-benzene soluble extractive content. Generally, the substances contained in the soluble extractive of benzene alcohol or benzene ethanol have properties that interfere with adhesion to wood (Sakuno and Moredo 1998). It is predicted that this case may occur because the extractive effect on the bonding strength may differ depending on the type of adhesive used. For example, in the study of Santoso *et al.* (2019) hot-water extraction treatment on raw materials was able to increase the internal bonding strength of particleboard bonded with citric acid. However, particleboard bonded with sucrose extraction treatment had a negative correlation with the bonding strength. It was suspected that in gambir tannin-based adhesive, the presence of alcohol-benzene extractive substances in tusam wood might have a positive effect on the bonding quality of its laminated composite board.

Based on the findings of the compressive shear strength test, it can also be inferred that the addition of an extender to gambir tannin glue remarkably reduced the bonding strength in this study. The findings of the HSD analysis demonstrate that the compressive shear strength of the adhesive without extender addition is significantly higher than the compressive shear strength of the glue with extender addition. The addition of an extender is intended to reduce adhesive cost and also increase its viscosity and bonding strength. However, in this study, the addition of an extender was not effective in increasing the bonding strength of TF adhesive. This is presumably due to the low tannin content of gambir leaves found in this study. The addition of flour extender in TF adhesive further reduced the amount of tannin substance contained in the adhesive. Tannins relatively give better adhesion strength than starch, which is present in flour. The addition of flour extender in tannin-based adhesive composition decreased the number of compounds with good adhesion ability in the adhesive. This led to the reduced adhesive bonding strength of adhesive; hence it also resulted in the lower compressive shear strength with the addition of an extender.

Conclusions

The gambir leaves condition that will be extracted, wood age, and addition of extender is known to significantly influence the compressive shear strength of the wood laminate product. The condition of fresh gambir leaves could produce a distinctly higher compressive shear strength

value. For the wood age factor, the value of the compressive shear strength tended to increase up to a certain age point (17-27 years), then the value of the shear strength decreased at the age of 28 years. Meanwhile, the addition of an extender in this study had a significant adverse effect on the compressive shear strength.

In general, gambir tannin has good potential to be applied as the wood adhesive for producing laminated tusam wood. This was proved with several gambir tannin formaldehyde adhesive characteristics in this study that could meet the PF standard. In addition, the value of bond strength of laminated tusam wood with gambir tannin formaldehyde adhesive in this study was also able to meet SNI and JAS standards. The value of bonding strength represented by the compressive shear strength produced by laminated products from tusam wood is around 8.46 – 109.30 kg/cm². The optimum level of extender added to Gambir TF adhesive found in this research was 0%, while the most suitable tusam wood age to produce Gambir TF laminated wood was considered to be 27 years old. Based on its specific gravity, gelation time, and solid content evaluation results, the reactivity of gambir-TF adhesive in this research has not reached its optimum level yet. Additional research is required to determine the treatment that can be applied during the production of gambir-TF adhesive to increase the tannin reactivity and overall features of gambir-TF adhesive. The treatment that can be done includes using different extraction methods to obtain better tannin reactivity or adding a particular compound to facilitate a better polymerization reaction.

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Author's Contribution

The three authors of this article (AS, ENA, and DAP) were the main contributors to the ideas and experimental designs carried out in this research. AS and ENA did all data collection and data analysis activities. AS, ENA, and DAP carried out the manuscript writing, revision, and finalization.

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Synergistic Effect of Alcohols, Ketone and Water on the Yield of Soluble Compounds and Tannins from the Barks of *Azadirachta indica* A. Juss. and *Moringa oleifera* Lam.

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Abstract

Investigations into the most efficient solvent or solvent mixtures, which yield substantial quantities of tannins for the production of adhesives for wood composite industries have been ongoing. However, knowledge of the synergistic effect of ketones, alcohols, and water on the yield of soluble compounds and tannins from plants is lacking. Using the Soxhlet extraction method, the synergistic effect of ketone, alcohol and water mixtures in the extraction of soluble compounds and tannins from the barks of *Moringa oleifera* and *Azadirachta indica* was examined. Ketone, water and alcohol did not have a definite synergistic effect on the yields of soluble compounds and tannins from the two plants. Mixtures of some solvents, as opposed to their individual solvents, recorded high yield of soluble compounds. The reverse was also observed. Water extract recorded the most substantial quantity of soluble compounds in *M. oleifera* while ethanol-methanol-water mixture (60:20:20) extracted the highest quantity of soluble compounds in *A. indica*. The mixtures containing either a ketone or the alcohols produced comparatively high tannin yields in *A. indica*. This trend was not so for *M. oleifera*. The efficiency of solvents to extract soluble compounds depended mostly on the species.

Keywords: Adhesive, lewis acid, phenol, plant extract, polar solvent, stiasny number, wood composite

Introduction

Synthetic glues such as urea-formaldehyde, resorcinol-formaldehyde and phenol-formaldehyde play important roles in the production of wood composites (Jetuah *et al.* 2001). National and international politics of oil-producing countries such as Russia, Iran, Saudi Arabia and the United States have led to the increase in the monetary value of crude oil components (resorcinol and phenol) used for glue production. This has necessitated the extraction of polyphenolic compounds such as tannins, from several plant parts (e.g. leaves, bark, fruits and stem) to replace the expensive crude oil phenol and resorcinol components (Readel *et al.* 2001) of adhesives. The demand for tannins for the production of adhesives has increased significantly due to their renewability and availability (Ugovsek *et al.* 2010). Many research has focused on the determination of efficient ways of extracting these tannins from plant parts. Conventionally, the extraction of tannins from plants is done with solvents such as methanol, acetone, water, ethanol, petroleum ether and sodium hydroxide or alkali (Darkwa and Jetuah 1996; Shi *et al.* 2021). The non-conventional method includes the use of ultrasound and microwave systems among others (Azwandia 2015).

Singh *et al.* (2014) asserted that the selection of solvents for extraction is an important phase for obtaining extracts with acceptable yields. Over the past decades, researchers have sought to determine the best solvents that improve the efficiency of extracting tannins. Darkwa and Jetuah (1996) reported 1% sodium hydroxide as a very efficient solvent for extracting tannins from *Rhizophora spp.* Antwi-Boasiako and Animapauh (2012) recorded a higher

amount of tannins for distilled water than 1% sodium hydroxide and thus concluded that water is highly efficient for tannin extraction from the leaves, bark and fruits of *Tetrapleura tetraptera*. Downey and Hanlin (2010) examined the relative effectiveness of aqueous mixtures of acetone and ethanol (in ratios ranging from zero to 100%) in the extraction of tannins. Acetone-water extracted more condensed tannins than ethanol-water. A solvent combination of acetone-water (80:20, v/v) used by Chavan and Amarowicz (2013) also extracted considerably higher amount of condensed tannins from beach pea (*Lathyrus maritimus L.*). According to Fraga-Corral *et al.* (2020), unlike the high polar hydrolysable tannins, condensed tannins have limited solubility in polar organic solvents such as water and would likely be efficiently extracted using low polar solvents including acetone.

Stefanelli *et al.* (2013), Singh *et al.* (2014) and Dailey and Vuong (2015) found that the extracting efficiencies of solvents were enhanced when they were mixed together. When solvents are combined, the range of soluble compounds, which they can extract broadens. Therefore, Arranz *et al.* (2009) explained that total plant polyphenols have often been underestimated due to the choice of extracting solvents and methods which could leave compounds behind. According to Fraga-Corral *et al.* (2020), the combination of water and either ethanol, sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), sodium bisulphite (NaHSO₃), or sodium sulphite (Na₂SO₃) improves extraction efficiency. Singh *et al.* (2014) found that the yield of extracts was higher for a mixture containing ethanol, ether and water compared to acetone only and ether only. Dailey and Vuong (2015) also indicated that the combination

of methanol, ethanol, acetonitrile and acetone with water resulted in a high yield of extracts compared to absolute ethanol, acetonitrile, water and acetone.

Extensive review of past research on tannin extraction revealed that most often, the solvent combinations have been made from either water and the alcohols or ketones and water. It is not clear from literature whether there would be an improvement in the extraction efficiencies of the solvents when two or more ketones only or alcohols only are combined. These combinations could be explored to determine their efficiencies in the extraction of tannins from plants.

Tannins, which are secondary compounds are readily available as they are widely distributed in plants (Antwi-Boasiako and Animapauh 2012). They are often found in the growth areas of trees, such as the secondary phloem and xylem and the bark (Hagerman 2002). *Moringa oleifera* and *Azadirachta indica* possess tannins in their barks which can be exploited for adhesive production (Prakash *et al.* 2002).

The objective of this research was to evaluate the efficiencies of ketones, alcohols and water and their combinations in extracting tannins from the barks of *Moringa oleifera* and *Azadirachta indica*. This was achieved by measuring the yield of soluble compounds and the amount of tannins (i.e. stiasny number) obtained from the barks of the two species by the solvents. Results from this research would provide the composite industry with knowledge on the possible range of solvent combinations, which can improve the amount of tannins obtained from plants for adhesive production.

Materials and Methods

Study Area

This study was conducted at the General Chemical Laboratory of the Faculty of Renewable Natural Resources (FRNR), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Bark samples of 33-year-old *A. indica* trees and 27-year-old *M. oleifera* trees were collected from the demonstration farms of the FRNR and the Department of Horticulture, KNUST. The sites are located in the moist semi-deciduous forest zone with an average altitude of 260 m and dominated by sandy loam soil. The zone has an average annual rainfall of 1,270 mm (Nolan and Twumasi 1992) and an average temperature of 25.9°C.

Sampling and Processing of Materials

The bark samples were collected from five randomly selected trees each of *A. indica* and *M. oleifera*. The samples were obtained from the stem of the trees at a distance of 4 feet from the ground along the boles of the trees, air-dried for 1 week, milled in the Wiley mill and sieved through a 0.5µm mesh. The milled samples were kept in airtight containers.

Preparation of Solvent Mixtures

Two alcohols [ethanol (Model Number: MFCD00003568) and methanol (Model Number: MFCD00004595)], one ketone [acetone (Model Number: MFCD00008765)] (all from VWR chemicals) and water were the solvents used for the extraction.

Table 1. Ratio of the individual solvents mixed together for the extraction of tannins

Solvent/Solvent mixtures ID	Ratio of the individual solvents in the mixture (%)				Total (%)
	Acetone	Ethanol	Methanol	Water	
A	25	25	25	25	100
B	60	20	20	0	100
C	0	60	20	20	100
D	20	0	60	20	100
E	20	20	0	60	100
F	0	0	0	100	100
G	100	0	0	0	100
H	0	100	0	0	100
I	0	0	100	0	100
J	50	50	0	0	100
K	50	0	50	0	100
L	50	0	0	50	100
M	0	50	50	0	100
N	0	50	0	50	100
O	0	0	50	50	100

These solvents were all miscible according to the solvent miscibility table. The ratios of these solvents in Table 1 were adopted from the ratios used by previous authors (e.g. Downey and Hanlin 2010; Antwi-Boasiako and Animapauh 2012) in similar works. About 250ml each of

fifteen (15) solvent mixtures at 90% concentration were prepared in a conical flask for the extraction process.

Tannin Extraction Process

Five grams of the milled samples from each species was weighed and loaded into the thimble of Glas-col Soxhlet

Extraction apparatus (Model: Glas-Col 100D RJ30424). The extraction solvent was placed in a distillation flask and heated to reflux until the solvent in the thimble chamber turned colourless. The extraction process was replicated three times for each species and solvent(s).

Qualitative Test for Tannins Obtained from the Extraction Process

The extracts obtained by the different solvents were subjected to 3 drops of iron (III) chloride (FeCl₃) solution and the colour changes were recorded and compared to the Axis Gear Standard Colour Chart (www.axisgear.ca), which served as a reference point. A blue to green or blue-black colouration indicated the presence of tannins (Darkwa *et al.* 1996). The test was repeated three times for each extract.

Quantitative Test for Soluble Compounds

Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents. The weights of five empty beakers were recorded and the beakers were filled with 5g of the milled samples and oven-dried at 105 ± 2°C for 24 h. The oven-dried milled samples were cooled in a desiccator for 1h and re-weighed. Oven-dried weight of the milled samples were determined from the difference between final weight of filled beakers and empty beakers. About 200ml of the extracts from the different solvents were oven-dried at 105 ± 2°C for 4 h, cooled in a desiccator for 1 h and weighed. The percentage yield of soluble compounds was determined by the formula (Antwi-Boasiako and Animapauh 2012);

$$\text{Yield (\%)} = \frac{\text{Weight of extracts after oven-drying}}{\text{Oven-dried weight of milled samples}} \times 100$$

The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate number) Contained in the Soluble Compounds. Stiasny number or formaldehyde precipitate number, which relates directly to the amount of tannins in the soluble compounds was determined. Stiasny number determination described by Wissing (1955) was adopted in quantifying the amount of tannins. A mixture of concentrated hydrochloric acid (250 ml) and Formaldehyde (40%) (500 ml) (1:2 v/v) was prepared in a volumetric flask. 50 ml of the extracts obtained from the extraction process was oven dried at 105 ± 2°C for 4 h, cooled in a desiccator for 1 h and the oven-dried weight recorded. Another 50 ml of the extracts were reacted individually with 10 ml of the Hydrochloric acid and Formaldehyde mixture to form a suspension. The suspension was then heated under reflux for 30 min. The suspension was allowed to cool to enable the formed precipitates to settle. The suspension was then filtered and the precipitate washed with hot water and oven-dried at 105 ± 2°C until constant weight. This process was replicated three times for each solvent extract. The stiasny number was determined by the formula (Antwi-Boasiako and Animapauh 2012);

$$\text{Stiasny number (\%)} = \frac{\text{Weight of precipitate after oven-drying}}{\text{Oven-dry weight of extract}} \times 100$$

Data Analysis

The data were subjected to ANOVA and LSD test to determine the significant differences ($p < 0.05$) between the amount of tannins extracted by the solvents and their mixtures.

Results

Qualitative Test for Tannins Obtained from the Extraction Process

The colour of the extracts from *A. indica* and *M. oleifera* before their reaction with iron (III) chloride varied from cinnamon to gold. After the reaction, there was a change in colouration, which ranged from black to army (Table 2). This indicated the presence of tannins in the extracts.

Quantitative Test for Soluble Compounds in *A. indica* and *M. oleifera*

Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents. More soluble compounds were obtained from *A. indica* than *M. oleifera* for all the solvents (Figure 1). In *A. Indica*, the mixture containing the alcohols (ethanol and methanol) and water (i.e., E-M-W) extracted the highest soluble compounds (97.96 ± 1.71%) followed by the ketone and water mixture (i.e., Acetone-Water) (69.01 ± 8.36%). The least yield of soluble compounds was obtained by the ketone and the alcohols mixture (i.e., A-E-M) (23.78 ± 0.34%). ANOVA showed significant differences between the solvents. For *M. oleifera*, water gave the highest yield of soluble compounds (39.39 ± 1.5%) followed by the mixture of ketone and water (i.e., Acetone-Water) (30.74 ± 3.79%) (Table 3). The difference was not significant ($p > 0.05$). The least yield of soluble compounds was obtained from the ketone/acetone only (3.29 ± 0.21%). ANOVA showed significant differences between the solvents.

Whereas the combination of the solvents resulted in a higher quantity of soluble compounds than the yield from the individual solvents in some instances, the reverse was also recorded for some mixtures. For instance, in *A. indica*, acetone, methanol and water independently produced a soluble compound yield of 31.44%, 34.06% and 34.54% respectively while their combination extracted 43.03% of soluble compounds. Similarly, in *M. oleifera*, while methanol and ethanol independently produced 11.58% and 7.97% of soluble compounds respectively, their mixture extracted up to 12.62% of these compounds. Nevertheless, while ethanol and water separately extracted 7.97% and 39.39% of soluble compounds from *M. oleifera*, their combination extracted only 13.53% of soluble

compounds. The mixture of three or more solvents did not improve the yield of soluble compounds in *M. oleifera*. Likewise, the yields of soluble compounds from acetone (31.44%), ethanol (43.51%) and methanol (34.06%) were higher than that from their mixture (23.78%) in *A. indica*. For example, while acetone, methanol and water independently extracted 3.29%, 11.58% and 39.39% of soluble

compounds respectively, their mixture extracted only 7.94% of soluble compounds.

With the exception of Ethanol-Water, Methanol-Water and A-E-M-W mixtures, all combinations containing water generally produced high yield of soluble compounds in *A. indica*. This was not so in *M. oleifera*.

Table 2. Colour change of extracts before and after reaction with Iron (III) chloride

Species	Solvent(s) used for the extraction	Extract colour before reaction with $FeCl_3$	Extract colour after reaction with $FeCl_3$	Inference
<i>A. indica</i>	Acetone (A)	Cinnamon	Black	Tannin present
<i>A. indica</i>	Methanol (M)	Brick	Forest	Tannin present
<i>A. indica</i>	Ethanol (E)	Brick	Forest	Tannin present
<i>A. indica</i>	Water (W)	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Ethanol	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Methanol	Brick	Forest	Tannin present
<i>A. indica</i>	Acetone-Water	Cocoa	Black	Tannin present
<i>A. indica</i>	Ethanol-Methanol	Brick	Black	Tannin present
<i>A. indica</i>	Ethanol-Water	Cinnamon	Black	Tannin present
<i>A. indica</i>	Methanol-Water	Brick	Black	Tannin present
<i>A. indica</i>	A-E-M-W	Cinnamon	Forest	Tannin present
<i>A. indica</i>	A-E-M	Brick	Black	Tannin present
<i>A. indica</i>	E-M-W	Brick	Black	Tannin present
<i>A. indica</i>	A-M-W	Brick	Forest	Tannin present
<i>A. indica</i>	A-E-W	Brick	Black	Tannin present
<i>M. oleifera</i>	Acetone	Gold	Forest	Tannin present
<i>M. oleifera</i>	Methanol	Army	Army	Tannin present
<i>M. oleifera</i>	Ethanol	Army	Army	Tannin present
<i>M. oleifera</i>	Water	Gold	Gold	Tannin present
<i>M. oleifera</i>	Acetone-Ethanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Acetone-Methanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Acetone-Water	Coffee	Forest	Tannin present
<i>M. oleifera</i>	Ethanol-Methanol	Citrus	Forest	Tannin present
<i>M. oleifera</i>	Ethanol-Water	Gold	Army	Tannin present
<i>M. oleifera</i>	Methanol-Water	Gold	Army	Tannin present
<i>M. oleifera</i>	A-E-M-W	Citrus	Forest	Tannin present
<i>M. oleifera</i>	A-E-M	Lemon	Army	Tannin present
<i>M. oleifera</i>	E-M-W	Tangerine	Army	Tannin present
<i>M. oleifera</i>	A-M-W	Lemon	Army	Tannin present
<i>M. oleifera</i>	A-E-W	Tangerine	Army	Tannin present

Table 3. Mean percentage yield of soluble compounds from *A. indica* and *M. oleifera*

Solvents/Solvent Mixtures	Yield of soluble compounds (%)	
	<i>A. indica</i>	<i>M. oleifera</i>
Acetone	31.44 ± 3.67 ^{abcdef}	3.29 ± 0.21 ^{abc}
Methanol	34.06 ± 1.88 ^{ghijklmn}	11.58 ± 0.81 ^{defghij}
Ethanol	43.51 ± 0.59 ^{opqrstu}	7.97 ± 1.33 ^{klmnopq}
Water	34.54 ± 1.59 ^{bgovwxyzab}	39.39 ± 1.50 [*]
Acetone-Ethanol	42.77 ± 0.52 ^{hpyvpθmδ}	6.61 ± 0.69 ^{alrstuvw}
Acetone-Methanol	42.55 ± 0.63 ^{iqwyΔμ&η}	8.76 ± 2.22 ^{ekrzyzβ}
Acetone-Water	69.01 ± 8.36 [*]	30.74 ± 3.79 [*]
Ethanol-Methanol	45.42 ± 6.9 ^{trpΔ12}	12.62 ± 2.08 ^{ixypθπ}
Ethanol-Water	33.29 ± 1.49 ^{cjx345}	13.53 ± 0.18 ^{γδ}
Methanol-Water	33.11 ± 0.33 ^{dky367}	15.94 ± 0.22 ^{ρδ}
A-E-M	23.78 ± 0.34 ^f	4.41 ± 0.02 ^{εηΔ1}
E-M-W	97.96 ± 1.71 [*]	9.38 ± 0.10 ^{houzθμz23}
A-M-W	43.03 ± 0.77 ^{mtam&18ε}	7.94 ± 0.05 ^{pvα&124}
A-E-W	40.60 ± 0.72 ^{nuβδγ2579ε}	9.50 ± 0.43 ^{iqwβητ34}
A-E-M-W	35.58 ± 1.17 ^{elszθμ4689}	6.71 ± 0.05 ^{bmsyΔμ&η}

Means with the same superscripts in the same column are not significantly different at 5% confidence level

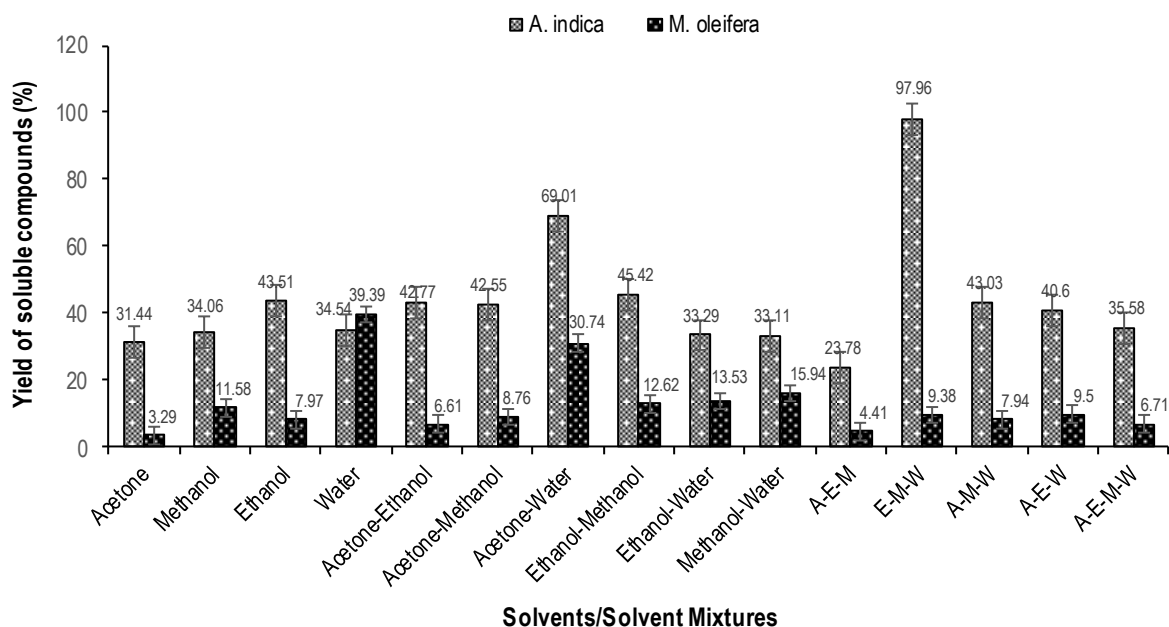


Figure 1. Mean percentage yield of soluble compounds obtained from *A. indica* and *M. oleifera*

The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate Number) Contained in the Soluble Compounds. The amount of tannins obtained from *A. indica* by all the solvents (i.e., $27.24 \pm 1.61 - 98.74 \pm 0.27\%$) were generally higher than those from *M. oleifera* ($1.61 \pm 0.2 - 34.56 \pm 3.4\%$) (Figure 2). The alcohols and water mixture (E-M-W) produced the highest yield of tannins in *A. indica* ($98.74 \pm 0.27\%$) while Ethanol only gave the highest yield ($34.56 \pm 3.4\%$) in *M. oleifera*. The least amount of tannins was obtained by water in *A. indica* ($27.24 \pm 1.61\%$) and Methanol-Water in *M. oleifera* ($1.61 \pm 0.2\%$) (Table 4).

The mixtures containing either a ketone (i.e., acetone) or the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield in *A. indica* (Figure 2). This

trend was not so for *M. oleifera*. With the exception of water only and, acetone, ethanol and methanol mixture (A-E-M), the individual solvents performed better than their various mixtures in *M. oleifera*. For *A. indica*, however, the mixtures largely produced high yield of phenols than the individual solvents except for some few mixtures involving either Methanol or Ethanol. The differences between the tannin yields of the solvents were significant for both species except between E-M-W and Acetone-Water in *A. indica* and, Acetone and Methanol, and Acetone and Ethanol in *M. oleifera*.

Table 4. Total amount of tannins (Stiasny number) contained in the soluble compounds from *A. indica* and *M. oleifera*

Solvents	Total amount of tannins (%)	
	<i>A. indica</i>	<i>M. oleifera</i>
Acetone	33.31 ± 2.51 ^a	30.63 ± 1.05 ^{abcde}
Methanol	77.53 ± 4.27 ^{bcdde}	33.44 ± 3.56 ^{afgh}
Ethanol	92.83 ± 0.88 ^{ghijkl}	34.56 ± 3.40 ^{bfi}
Water	27.24 ± 1.61 ^a	3.94 ± 0.83 ^{iklm}
Acetone-Ethanol	97.87 ± 0.77 ^{mnopq}	24.03 ± 1.54 ^{no}
Acetone-Methanol	93.16 ± 1.15 ^{qrstuv}	12.48 ± 0.53 ^{pqr}
Acetone-Water	98.03 ± 0.27 ^{hnnwxy}	27.20 ± 2.85 ^{gnst}
Ethanol-Methanol	70.48 ± 5.88 ^{bz}	8.94 ± 1.44 ^{juvw}
Ethanol-Water	82.38 ± 4.60 ^{coβ}	3.16 ± 0.64 ^{kuxy}
Methanol-Water	71.27 ± 1.42 ^{dz}	1.61 ± 0.20 ^{lz}
A-E-M	88.44 ± 0.24 ^{isβyp}	43.90 ± 0.31 [*]
E-M-W	98.74 ± 0.27 ^{otwθπ}	7.43 ± 3.58 ^{mnyz}
A-M-W	97.72 ± 0.32 ^{kpuvθδ}	24.49 ± 4.32 ^{dos}
A-E-W	93.28 ± 0.43 ^{qvypπδ}	31.50 ± 1.14 ^{ehit}
A-E-M-W	82.62 ± 1.17 ^{eoγ}	14.97 ± 2.29 ^{qv}

Means with the same superscripts in the same column are not significantly different at 5% confidence level.

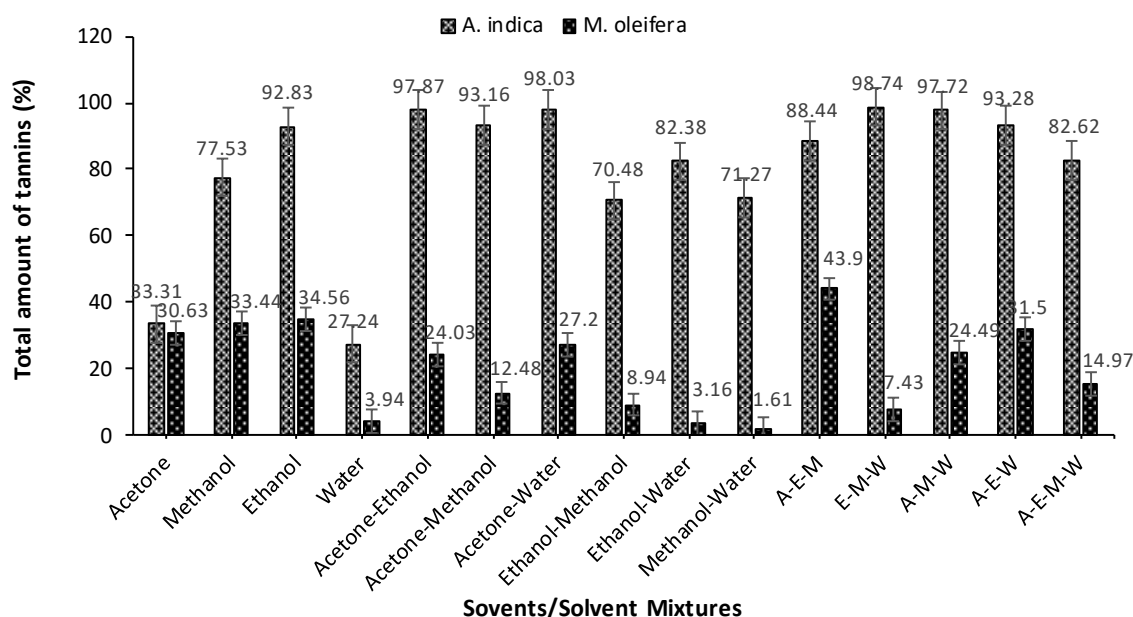


Figure 2. Total amount of tannins (Stiasny number) contained in the soluble compounds from *A. indica* and *M. oleifera*

Discussions

Qualitative Test for Tannins Obtained from the Extraction Process

Tannins are phenols, which are tested qualitatively using ferric salts. They form compounds with ferric salts such as iron (III) chloride, which possess a metal centre bound to ligands (atoms, ions or molecules). The ligands donate electrons to the metal (Fe^{3+}) and this transfer of charges, gives a very strong spectral signal in the visible range of the electromagnetic spectrum. Phenols contain hydroxyl groups (hydrogen and oxygen) and are relatively strong acids as iron (III) chloride which is a Lewis acid. According to Sarhan and Bolm (2009), all oxygen containing

compounds act as bases in the presence of Lewis acids. This results in an acid-base reaction, with the formation of a precipitate and ligand exchanges, which causes a colour change.

In determining the presence of tannins in plant extracts Darkwa and Jetuah (1996) and Lysyuk (2011) explained that when iron (III) chloride is added to the extracts and the colour of the extract changes to blue-black or green, it indicates that tannins are present. When 3 drops of iron (III) chloride were added to the extracts from the barks of *Moringa oleifera* and *Azadirachta indica*, their colours changed to shades of green and black (Table 2). The colour change could be attributed to the ligand exchanges between the phenols and iron (III) chloride. Dhawan and Gupta (2017) similarly observed these colour

changes with *Datura metel* leaves extract after reaction with iron (III) chloride, and concluded that the extract contained tannins.

The difference in the colours of the extracts after their reaction with the Lewis acid represents the variations in the amount of tannins found in the plants. Deep green to black colouration shows a high tannin content while light blue to green or no shade of green indicates a low or no tannin content (Antwi-Boasiako and Animapauh 2012). *A. indica* bark gave deeper colour shades of forest (green with black undertone) and black than *M. oleifera* where the extract colours were gold, and mostly army (light shade of green). Thus, *A. indica* bark extracts were expected to have a higher tannin content than *M. oleifera*.

Quantitative Test for *A. indica* and *M. oleifera*

Percentage Yield of Soluble Compounds Obtained from *A. indica* and *M. oleifera* by the Solvents. The yield of soluble compounds from plant parts is a measure of the efficiency of the extracting solvent(s) (Antwi-Boasiako and Animapauh 2012; Boadu *et al.* 2018). Plant extracts contain carbohydrates, alkaloids, terpenoids and phenols (Hagerman 2002); high yield of soluble compounds means there is a high percentage of these components in the extract of the plant. In the present study, more soluble compounds were obtained from *A. indica* than *M. oleifera* for all the solvents. These compounds have varying polarities (Akowuah *et al.* 2005) and their dissolution in specific solvents depends, among others, on the polarity of the solvent (Cuong *et al.* 2020). According to Zuo *et al.* (2002), polar substances easily dissolve in polar solvents and vice versa. The solvents used in the extraction of soluble compounds from *A. indica* and *M. oleifera* had an increasing order of polarity as acetone < ethanol < methanol < water (Tan and Kassim 2011). The yield of soluble compounds in *M. oleifera* also increased as acetone (3.29%) < ethanol (7.97%) < methanol (11.58%) < water (39.39%). Thus, more of the soluble compounds in *M. oleifera* were possibly polar and therefore dissolved in the most polar of all the solvents (i.e., water). Similarly, methanol extraction resulted in a higher yield of soluble compounds from *Datura metel* leaves than acetone (Dhawan and Gupta 2017). In *A. indica*, however, ethanol, which is less polar than water had a high yield of soluble compounds (43.51%) than methanol (34.05%) and distilled water (34.53%). Thus, unlike *M. oleifera*, more of the soluble compounds in *A. indica* could have been less polar and were, therefore, likely attracted to ethanol than the highly polar solvents such as water.

Chavan and Amarowicz (2013) explained that the yield of soluble compounds is often improved by the use of multiple solvents for extraction. This is due to the synergistic strength of the solvents produced by the mixture. This assertion was found to be partially true in the current study since not all the solvent mixtures improved the soluble compound yield from *A. indica* and *M. oleifera*. This work

has found that the type of plant species contributes to the extracting efficiencies of the solvents and their mixtures.

Mixtures containing water extracted the highest quantities of soluble compounds in both species. Darkwa and Jetuah (1996) explained that water is able to dissolve a large range of compounds including the low molecular weight carbohydrates compared to the other solvents. Its combination with the alcohols and ketone broadened the range of compounds that could be dissolved by the mixture. Hence, the high yield of soluble compounds recorded for mixtures containing water.

The Amount of Tannins (Stiasny Number or Formaldehyde Precipitate Number) Contained in the Soluble Compounds. The Stiasny number of extracts is an estimate of the amount of reactive tannins that get attached to formaldehyde in the process of quantifying the phenols (Simon *et al.* 1992; Paridah 2002; Pandey and Rizvi 2009). Thus, a high stiasny number of a plant extract means that most of the soluble compounds in the extract that got attached to formaldehyde are tannins (Hagerman, 2002; Antwi-Boasiako and Animapauh 2012). For all solvents, more tannins were obtained from *A. indica* (i.e., $27.24 \pm 1.61 - 98.74 \pm 0.27\%$) than *M. oleifera* ($1.61 \pm 0.2 - 34.56 \pm 3.4\%$). For the single solvents, Darkwa and Jetuah (1996) recorded higher tannin content (74%) in the soluble compounds extracted from *Rhizophora spp.* with water than those extracted with ethanol (61%) and petroleum ether (3.2%). On the contrary, water produced lower amount of tannins (27.24% in *A. indica* and 3.94% in *M. oleifera*) in the present study than ethanol (92.83% and 34.56% respectively). The mixtures containing either a ketone (i.e., acetone) or the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield in *A. indica*. Thus, although water extracted more soluble compounds in both species, smaller percentage of these compounds were tannins. Tannins have low polarity (Tian *et al.*, 2009) and the condensed type has limited solubility in highly polar organic solvent such as water (Fraga-Corral *et al.* 2020). The low polarity of tannins probably restricted its dissolution in water, which has high polarity compared to the low polar solvents such as ethanol and acetone (Zuo *et al.* 2002).

Downey and Hanlin (2010) found acetone-water mixture more effective than ethanol-water mixture for the extraction of condensed tannins from grape skin. Chavan and Amarowicz (2013) also reported higher quantities of tannins from beach pea with acetone-water mixture than ethanol-water and methanol-water mixtures. Our results agree with the observation by these authors. With the exception of mixtures involving either methanol or ethanol, the solvent mixtures generally improved the stiasny numbers of extracts from *A. indica*. For example, while the alcohols and water mixture (E-M-W) produced the highest yield of tannins ($98.74 \pm 0.27\%$), water only produced the least amount of tannins ($27.24 \pm 1.61\%$). In *M. oleifera*, however, more tannins were extracted by the individual solvents (e.g. $34.56 \pm 3.4\%$ by ethanol only) than their

various mixtures (e.g. $3.16 \pm 0.64\%$ by ethanol-water mixture) except water only and, acetone, ethanol and methanol mixture (A-E-M). Therefore, a mixture of solvents will not always produce a synergistic effect on the yields of soluble compounds and tannins from plants.

According to Yazaki and Collins (1994) and Ferreira *et al.* (2008), at least 65% of tannins must be obtained from extracts of plants in order to recommend that plant as a raw material source for commercial wood adhesive production. With the exception of extracts from acetone only and water only in *A. indica*, the quantities of tannins from the other solvent extracts were above 65%, which meets the threshold for adhesive production. Thus, extracts from *A. indica* would be most useful to commercial adhesive producers. In *M. oleifera*, all the solvent extracts did not contain the minimum amount of tannins required for adhesive production. However, based on the recommendation of Ferreira *et al.* (2008), tannins extracted with acetone, methanol, ethanol, Acetone-Ethanol-Methanol and Acetone-Ethanol-Water mixtures can serve as tannin mordants.

Conclusions

The present work investigated the synergistic effect of ketone, water and alcohols on the yield of soluble compounds and tannins from the barks of two tropical plants, namely, *Azadirachta indica* and *Moringa oleifera*. The conclusions from the work are as follows.

Mixtures of ketone, water and alcohols will not always produce a positive synergistic effect on the yields of soluble compounds and tannins from plants. The extracting efficiencies of the solvents depended mostly on the species. While water extracted the highest quantity of soluble compounds in *M. oleifera*, ethanol-methanol-water mixture extracted the highest quantity of soluble compounds in *A. indica*.

Although water extracted more soluble compounds than all other solvents in *M. oleifera*, a small percentage of these compounds were tannins. The mixture containing ketone (i.e., acetone) and the alcohols (i.e., methanol and ethanol) produced comparatively high tannin yield even though it extracted low quantities of soluble compounds. In *A. indica*, the least yield of soluble compounds was obtained by the ketone and the alcohols mixture (i.e., A-E-M) while mixtures containing either a ketone or the alcohols produced comparatively high tannin yield.

Unlike *M. oleifera* extracts, *A. indica* extracts from all the solvents except acetone only and water only would be most useful to commercial adhesive producers since the quantities of tannins obtained from them were higher than the minimum threshold (65%) required by the adhesive industry.

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Biological Resistance of Jabon Wood Against Subterranean and Drywood Termites after Combined Impregnation and Compression Treatment

Jamaludin Malik, Adi Santoso, Jasni, and Barbara Ozarska

Abstract

This study investigated Jabon wood [*Anthocephalus cadamba* (Roxb) Miq] impregnated with natural phenolic resin from Merbau extractives (ME) and subjected to hot compression treatments. The resistance of this wood to subterranean termites (*Coptotermes curvignathus* Holmgren) and drywood termites (*Cryptotermes cynocephalus* Light) was tested. The types of pre-treatments utilized were impregnation, repeated-impregnation, hot compression, and a combined treatment of impregnation and hot compression. The impregnation pre-treatment used crude ME and selected polymerized ME (PME22 and PME33). The resistance evaluation was based on the weight loss of the samples according to the Indonesian standard of SNI 7207. Untreated samples of jabon were classified as class V (susceptible) against subterranean termites and class IV (non-resistant) against drywood termites. Impregnation using only ME improved the resistance of Jabon wood against subterranean termite from class V to class IV. Against drywood termites, it changed from class IV to III and II. The resistance class of III against subterranean termites resulted from the treatments of most types of polymerized ME. Thus, it can be concluded that the extractives contributed greatly to the protection of a less durable wood species against termite attack. Merbau extractives also distinctly improved the resistance of jabon wood from class IV (non-resistant) to class II (resistant).

Keywords: resistance, subterranean termite, drywood termite, impregnation, hot compression.

Introduction

Although there are differences in opinion as to whether wood preservation should be included in the field of wood modifications, it can be found in many studies that biocidal treatments improve wood durability. Taking environmental awareness into account, Homan and Jorissen (2004) and Hill (2006) proposed the exclusion of biocidal treatments and thus the separation of wood preservation from wood modification, stating that it should not involve the production of a product that contains toxic residues. However, wood preservation does meet at least one of the criteria of wood modification; according to Hill (2006), wood modification may be used to bring about an improvement in decay resistance and dimensional stability, reduce water sorption, improve weathering performance, etc. Wood modification alters the properties of materials to prevent the loss of enhanced performance during the lifetime of a wood product. For example, using conventional technologies, the decay resistance of wood can be increased by the application of wood preservatives (e.g., boron, creosote, etc.) (Hill 2006; Coggins 2008).

Recently, wood preservation has changed in regards to biocide toxicity, which can be attributed to increasing legislative environmental pressure and awareness (Villanueva *et al.* 2013). Consequently, many bioactive termite control compounds have been withdrawn from the market in the past decade due to environmental or toxicity concerns (Little *et al.* 2010). Therefore, non-biocidal and environmentally friendly alternative modification methods, such as the chemical or thermal modification of the wood cell wall, are in high demand (Hill 2006).

There have been various studies on eco-friendly treatments to improve resistance against biological attack. He *et al.* (2011) treated fast-growing poplar wood (*Populus euramericana*) from a 15 year-old plantation by impregnation using styrene (ST) and a combined solution of glycidyl methacrylate (GMA)-ST at a 1:4 molar ratio. Specimens to be tested for termite resistance were cut to the dimensions of 6.4 mm (L) by 25 mm (T) by 25 mm (R). Before the impregnation, the samples were oven-dried at 103 ± 2 °C for 24 h. Impregnation was conducted through vacuum at 0.05 MPa for 15 min, 0.1 MPa for 30 min, and then immersed in the solution at ambient pressure for 3 h. The untreated and treated wood specimens were exposed to subterranean termites (*Coptotermes formosanus*) in containers at a normal environmental temperature (25 ± 2 °C). The results showed that ST-treated polymer infused wood (PIW) exhibited 5.4 times higher resistance to termites than untreated wood; GMA-ST-treated PIW showed 9.3 times higher resistance. The weight losses of ST-treated and GMA-ST-treated PIWs were 4.6 and 2.7%, respectively.

Unsal *et al.* (2009) studied the resistance of solid wood specimens from Scotch pine (*Pinus sylvestris* L.) with dimensions of 250 mm by 500 mm by 18 mm against *Reticulitermes flavipes* Kollar (Eastern subterranean termites). Solid panels were first hot-pressed at 120 or 150 °C and a pressure of either 5 or 7 MPa for 60 min. For the termite test, five specimens (20 mm by 20 mm by panel thickness) were used for each treatment group. The specimens were placed in a glass container with moist sand and 1 g of *R. flavipes*. The containers were maintained at 25 °C and 80% relative humidity (RH) for four weeks. The compression treatment resulted in the improved resistance

of wood against termite attacks. Samples treated with compression at 7 MPa and 120 °C gave higher resistance than the other treated samples and the controls.

Other studies conducted on various wood species using eco-friendly preservatives also revealed a bio-resistance improvement against termite attacks. A phenolic resin (PF) can enhance the decay and termite resistance of particleboard (Kajita and Imamura 1991). Impregnation with dimethyloldihydroxy-ethyleneurea (DMDHEU) can prevent the sapwood of Scots pine (*Pinus sylvestris*) from weight losses in contact with subterranean termites (Militz *et al.* 2011). Little *et al.* (2010) showed that termites avoid wood that contains relatively high levels of synthetic or natural antioxidants that are benign to humans. Consequently, the reason why some heartwoods are naturally resistant to termites may be that they contain relatively high levels of phenolic extractives, which have antioxidant properties.

In Indonesia, termite attack is the most important problem in wood utilization because 85% of wood species in the country are susceptible to biodeterioration, and only 15% are classified into durable classes (class I and II) (Martawijaya 1996; Martawijaya and Barly 2010). There are approximately 200 species of termites living in forest, farming, estate, settlement, and business areas (Nandika 2015). Financial losses resulting from termite attacks have continuously increased from IDR 1.67 billions in 1995 to IDR 8.68 billions in 2015 (Rakhmawati 1996; Nandika 2015).

This paper examined the effect of impregnation treatment on jabon wood (*Anthocephalus cadamba* (Roxb) Miq), using natural phenolic resin from Merbau extractives (ME) combined with hot compression treatments. The resistance of treated vs. untreated wood was tested against subterranean (*Coptotermes curvignathus* Holmgren) and drywood (*Cryptotermes cynocephalus* Light) termites. Because jabon wood is of the lowest class of durability (class V), it has limited uses (Hidayat 2012; Martawijaya *et al.* 1989).

Materials and Methods

Wood Sample Preparation

Five-year-old jabon wood was harvested and sawn. Samples were prepared only from sapwood with various dimensions. All samples were kiln-dried to 12% moisture content and then grouped and coded based on the pre-treatment given. The sample dimensions followed the BSN (2014) standard for wood resistance testing against wood destroying organism with the dimensions of 25 mm (L) by 25 mm (T) by 5 mm (R) and was used for the impregnation and testing against subterranean termites. Samples with the dimensions of 50 (L) mm by 25 mm (T) by 25 mm (R) were prepared for impregnation and testing against drywood termites. Four replicates of the samples were made for each treatment.

The types of pre-treatments were impregnation, repeated-impregnation, hot compression, and a combined treatment of impregnation and hot compression. The impregnation pre-treatment used crude Merbau extractives (ME) and selected polymerized Merbau extractives (PME22 and PME33). The Merbau extractives were obtained from boiling Merbau wood sawdust in hot water (70 – 80 °C) (Malik *et al.* 2016). Samples with the impregnation treatment were divided into three groups according to how many times the treatment was done (one, two, or three impregnations) and were coded accordingly, as shown in Table 1.

Pre-Treatment by Impregnation

Impregnation treatment was conducted in a vacuum-pressure vessel (a vertical container with 6" in diameter; non-lethal service; local made) as a pre-treatment for jabon wood samples before biologically testing their termites. The samples were placed into open-top containers in a vacuum-pressure unit, which was connected by a hose to the container with liquid of polymerized Merbau extract (Fig. 1a). The tube containing the samples was vacuumed using the compressor at a power of 0.1 kg/cm² for 30 min. The vacuum was then released, and the impregnation liquid filled the container, which caused the liquid level to be 10 cm higher than the submerged wood samples. Pressure was then applied and maintained at 15 kg/cm² for 1 h.

Impregnation was carried out 2 to 3 times to investigate the effect of replication on termite attacks. The second and third impregnations were applied with the same procedure. Each impregnation was done after the samples from the previous impregnation were conditioned at room temperature (28 °C) to reach a constant weight (Fig. 1b). All specimens were drained and stored in a conditioning room at 12% moisture content until a constant weight was obtained.

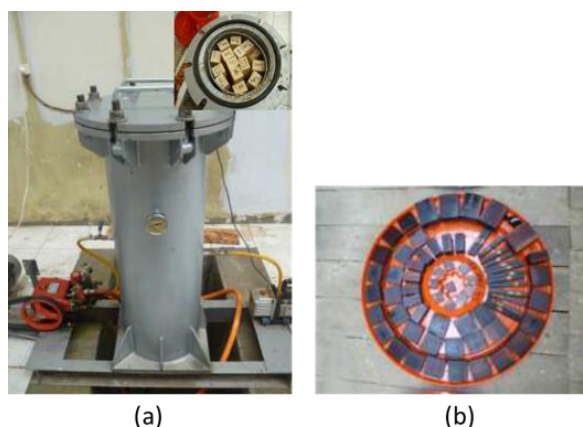


Figure 1. Treating samples in a vacuum pressure vessel (a) and conditioning them (b).

Pre-Treatment by Hot Compression

Hot compression pre-treatment was conducted in an open machine system (ex Becker and Van Hullen, Germany). The wood samples, 400 x 400 mm, were placed on the working table or hot plate. The maximum pressure force was 64 T, and the maximum temperature 200 °C. In this experiment, the working pressure applied to the samples was 10 kg/cm² for 20 min at a temperature of 150 °C until the compressed samples reached a selected compression ratio (CR), i.e. 33% and 50%.

Termite Tests

The treated and untreated wood samples were exposed to subterranean termites (*Coptotermes curvignathus*, Holmgren) drywood termites (*Cryptotermes cynocephalus*, Light). The test procedures were according to the Indonesian National Standard for wood resistance testing against wood destroying organisms (BSN 2014).

Exposure to Subterranean Termites. Ten samples from each pre-treatment were placed on the base of a cylindrical test container/glass jar (100 mm diameter) in a standing position. Next, 200 g of unsterilised sand with a moisture

content (MC) of approximately 7% (under water holding capacity) and 200 subterranean termites were added to the container (Fig. 2). Each container was prepared for each pre-treatment. All containers were placed in a dark room at normal environmental temperatures, ranging from 28 to 32 °C, for 4 weeks. If the MC of the sand decreased more than 2%, water was sprayed into the jar to keep the current sand MC equal to the initial MC (approximately 7%). The termite test containers were examined every week at the same time, and the dead termites were removed and tallied. The living termites in each container were counted after 4 weeks of exposure to subterranean termites. At the end of the test period, all of wood samples were washed, dried at 60 ± 2 °C for 48 h, and weighed. The weight loss of the specimens was then calculated using Eq (1).

$$P = \frac{W1-W2}{W2} \times 100 \quad (1)$$

where P was the mass (weight) loss (percent), W1 was the weight of the sample before feeding (g) and W2 was the weight of the sample after feeding (g).

Table 1. The Code of Samples Used for Termite Test

Code	Treatment
A	Untreated samples as the control
B	Impregnated using ME for 1x
C	Impregnated using ME for 2x
D	Impregnated using ME for 3x
E	Impregnated using PME22 for 1x
F	Impregnated using PME22 for 2x
G	Impregnated using PME22 for 3x
H	Impregnated using PME33 for 1x
I	Impregnated using PME33 for 2x
J	Impregnated using PME33 for 3x
K	Combined treatment of impregnation using PME 33 (1x) + Hot pressing by 33% CR
L	Combined treatment of impregnation using PME 33 (1x) + Hot pressing by 50% CR
M	Impregnated using ME (1x)+ Hot press by 33% CR
N	Impregnated using ME (1x)+ Hot press by 50% CR
O	Hot press by 33% CR without impregnation
P	Hot press by 50% CR without impregnation

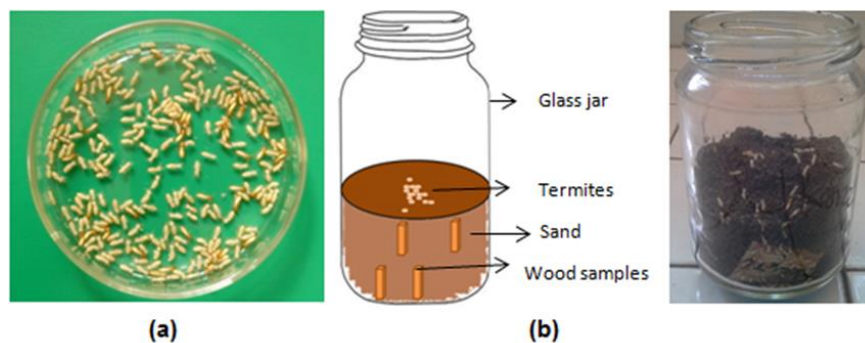


Figure 2. Subterranean termites (a) and the containers used in the investigation.

Table 2. Resistance Classes of Woods to Subterranean Termite (BSN 2014)

Class	Weight Loss (%)	Class of Resistance
I	< 3,5	Very resistant
II	3.51 – 7.52	Resistant
III	7.53 – 10.96	Moderately resistant
IV	10.97 – 18.94	Non-resistant
V	> 18.94	Susceptible

The resistance classes of examined jabon wood samples were determined based on weight loss, the number of living termites, and the degree of attack (Table 2 and Table 3) (BSN 2014).

Table 3. Degree of Subterranean Termites Attack (BSN 2014)

Sample Condition	Degree of Attack (% damage)
No damage on surface area	0 – 5
Slightly attacked	5 – 15
Moderately attacked	16 – 35
Heavily attacked	36 – 50
Very heavily attacked	> 50

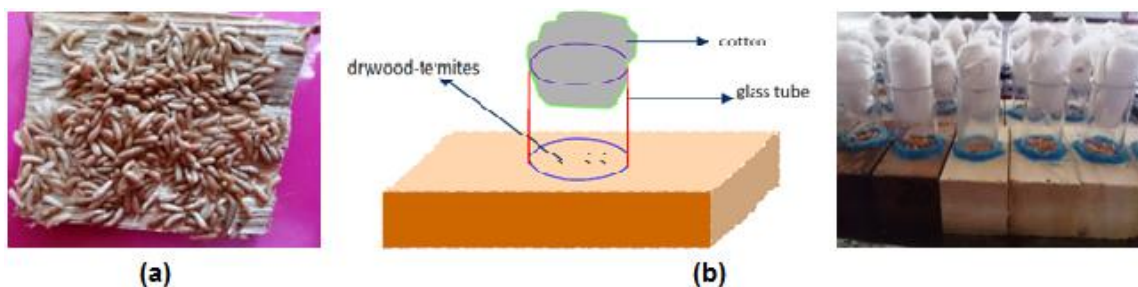


Figure 3. Drywood termites (a) and feeding method for its attack investigation (b)

Table 4. Resistance classes of wood to drywood termite (BSN 2014)

Class	Weight loss (%)	Class of resistance
I	< 2	Very resistant
II	2 – 4,4	Resistant
III	4.45 – 8.2	Moderately resistant
IV	8.3 – 28.1	Non-resistant
V	>28.1	Susceptible

Exposure to Drywood Termite. A glass tube (diameter, 18 mm; height, 30 mm) was placed vertically on the wide side of each wood sample. A total of 50 healthy, active worker termites were placed inside the tube, and the top of the tube was covered by cotton (Fig. 3). All samples were stored in a dark room for 12 weeks. At the end of the test period, the wood samples were washed, dried at 60 ± 2 °C for 48 h, and weighed. The weight loss was calculated using Eq. (2).

$$P = \frac{W_1 - W_2}{W_2} \times 100 \quad (2)$$

where W_1 was the oven-dried weight of the specimen before feeding and W_2 was the oven-dried weight of the specimen after feeding. Resistance classes were determined based on weight loss and the number of living termites (Table 4), whereas the degree of attack was determined using the same method employed for subterranean attack. The resistance classes of examined

jabon wood samples were determined based on weight loss, the number of living termites, and the degree of attack (Table 2 and Table 3) (BSN 2014).

Data Analysis

The weight loss data obtained from both investigations of the samples exposed to drywood and subterranean termites were recorded according to the type of treatments. The wood samples were then classified into the resistance class according to BSN (2014). To determine the differences in weight loss and the living termites, an analysis of variance was conducted. A further analysis of Duncan post difference test was done to point out the differences between the treatments.

Results and Discussion

Resistance Against Subterranean Termites

Table 4 presents weight loss, termite mortality, and the degree of attack of treated and untreated jaboron wood used to determine its resistance against subterranean termites. To determine the weight loss and the living termites, an analysis of variance was conducted. The results revealed that there was a significant difference in weight loss ($F_{calc} = 21.06 > F_{table} = 1.84$) and living termites ($F_{calc} = 28.49 > F_{table} = 1.84$). Furthermore, the Duncan post difference test was carried out to determine the differences in the effect of each pre-treatment to the weight loss.

Table 4 and Fig. 4 show that the highest weight loss of 19.12% occurred on the untreated (control) samples. Regarding the class of resistance, the untreated samples (A) belonged to class V, as the weight loss was more than 18.94% (BSN 2014). This result was in accordance with the Indonesian wood database, which classified the durability of jaboron into class V. It is the lowest class of resistance against termite attack. The highest weight loss of the control samples was followed by treatment B (impregnated once using Merbau extractive (ME)), treatment C (impregnated twice using Merbau extractives, ME), and treatment D (impregnated three times using ME), with weight losses of 16.61%, 16.41%, and 15.25%, respectively. All of these samples were classified as class IV (non-resistant to termite attack).

In contrast, the lowest weight loss was obtained in the samples with treatment G (impregnated three times using

polymerized ME (PME) type 22) by 4.40%, treatment L (impregnated once using PME 33 and hot pressing with 50% CR), treatment M (impregnated once using ME + hot press by 33% CR), and treatment K (impregnated once using PME 33 + hot press by 33% CR), with weight losses of 5.20%, 6.5%, and 6.52%, respectively. All four of these treatments resulted in resistance class II (resistant to termite attack). Other impregnated samples using PME (treatments E, F, H, I, J, N, O, and P) improved the resistance against termites to class III (moderately resistant to termite attack). This result was similar to the result of a study in which jaboron was impregnated with methyl methacrylate (MMA). In that case, the resistance was improved from class V to II (Hadi *et al.* 2015). In general, based on weight loss, the samples with all different treatments (B through P) showed higher resistance against subterranean termite attack, with improvement from class V (non-resistance) to class IV (treatments B, C, and D), III (treatments E, F, H, I, J, N, O and P), and II (treatments G, K, L, and M).

In terms of resistance or durability class, according to Seng (1990), class V means that the wood is susceptible to termites or other destroying organisms' attack. Consequently, the wood is very quickly damaged or possesses low durability if it is constantly in contact with moist ground. Even if the wood is sheltered and has no moist ground contact, the durability of the wood would still be short. However, if the timber is well maintained and periodically painted, the lifetime of the wood can reach 20 years, which also depends on the species.

Table 5. Weight loss, resistance, and the degree of attack of subterranean termites (*Coptotermes curvignathus* Holmgren) against Jaboron wood.

Treatment	Weight Loss (%)	Resistance Class	Mortality			Degree of Attack (%)
	X ± Sd *		%	X ± Sd *		
A (Control)	18.12 ± 2.30 a	V	22.38	28.17 ± 2.69 d	37.50	
B	16.61 ± 1.77 ab	IV	25.75	30.47 ± 1.97 cd	36.25	
C	16.40 ± 0.99 ab	IV	24.88	29.91 ± 1.14 cd	36.25	
D	15.25 ± 1.61 bcde	IV	29.25	32.73 ± 1.37 c	32.50	
E	8.79 ± 3.27 cde	III	38.25	38.20 ± 1.09 b	17.50	
F	8.95 ± 2.13 cde	III	35.5	36.54 ± 2.82 b	23.75	
G	4.40 ± 1.44 g	II	50.75	45.43 ± 2.49 b	17.25	
H	8.88 ± 2.82 cde	III	36.88	37.36 ± 2.74 a	22.50	
I	9.59 ± 1.75 cd	III	38.75	38.56 ± 1.95 b	22.50	
J	9.85 ± 0.61 c	III	37.25	37.60 ± 2.05 b	25.00	
K	6.52 ± 1.20 defg	II	52.00	46.15 ± 2.00 b	18.75	
L	5.20 ± 1.76 fg	II	52.13	46.22 ± 0.75 a	16.50	
M	6.15 ± 2.37 efg	II	40.38	39.44 ± 2.08 b	17.50	
N	7.87 ± 0.42 cdef	III	40.88	39.38 ± 1.71 b	18.00	
O	7.88 ± 2.37 cdef	III	38.50	38.35 ± 1.38 b	20.00	
P	9.93 ± 1.88 c	III	36.88	37.36 ± 2.72 b	18.25	

Remarks: A through P refers to Table 1; (*) The mean value followed by the same letter means no significant difference ($p = 0.05$) was determined based on Duncan post hoc test; Sd = Standard deviation.

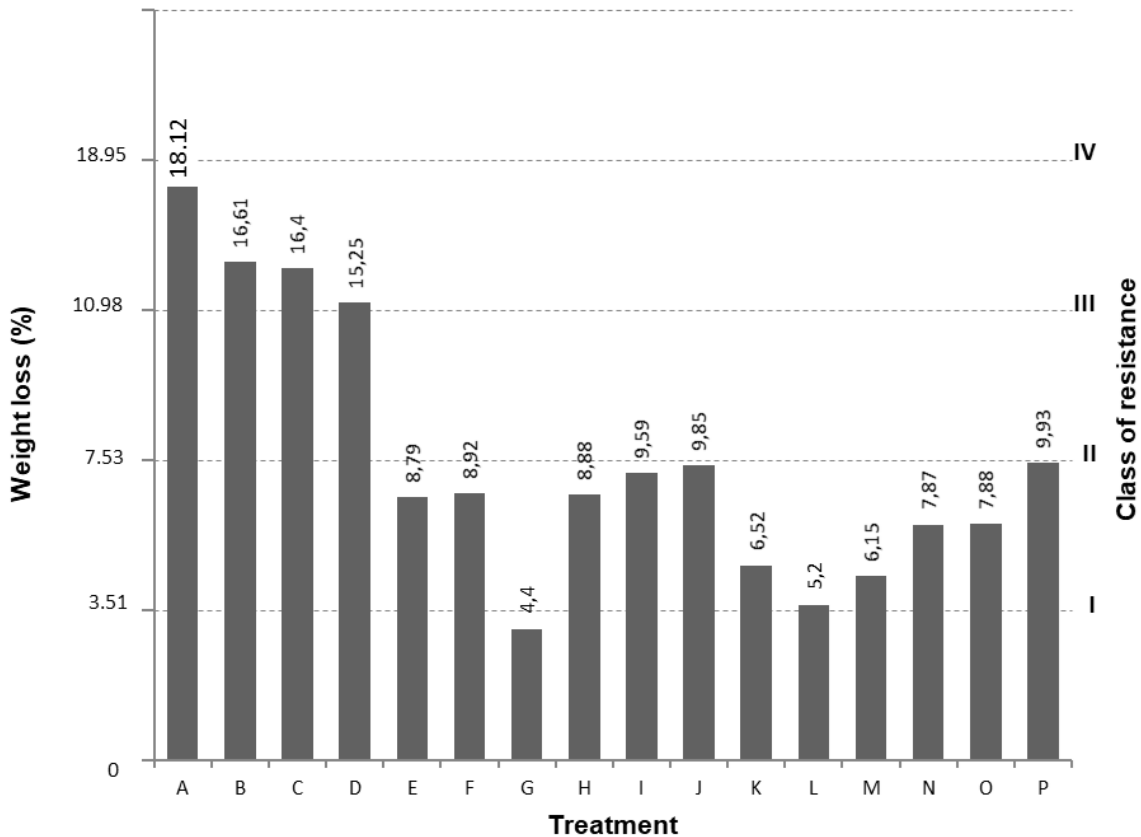


Figure 4. Weight loss of treated and untreated jabon wood due to subterranean termite attack.

Besides weight loss, mortality and the degree of attack are also used to determine wood resistance against termite attack. Based on the mortality, the more termites that are dead during the test, the more resistant the wood samples are against termite attack. The highest mortality rate occurred on the samples with treatments L, K, and G, with the mortality of 52.13%, 52.0%, and 50.75%, respectively (Table 4). Other treatments resulted in a mortality rate ranging from 24.88% to 40.88%. The mortality of the control samples was 19.12%. To determine which treatment was most efficient in protecting jabon wood from subterranean termite attack, Table 4 presents Duncan's multiple range test, which showed the same resistance class due to the same weight loss percentage. The treatments that improved wood durability to class II were G, K, L, and N. However, the samples of K, L, and N treatments needed hot compression treatment. Treatment G (impregnation using PME22 three times) could be considered the preferred treatment, as it is the easiest and simplest treatment and is a more affordable treatment than others (K, L and M) that resulted in the same resistance class against subterranean termites.

Resistance Against Drywood Termites

Similar to resistance against subterranean termites, the parameters used to evaluate the resistance against drywood termites were weight loss and mortality. The results are presented in Table 5 and Fig. 5. Analysis of variance was done to determine the differences in weight loss as well as mortality for different treatments. The results showed that there were significant differences for weight loss amongst treated and untreated samples ($F_{calc} = 4.47 > F_{table} = 1.84$) as well as for mortality ($F_{calc} (2.87) > F_{table} = 1.84$). Further analysis by the Duncan post hoc test was carried out to determine the differences in weight loss as well as mortality among the treatments.

Table 6 and Fig. 5 revealed that the highest weight loss occurred in group A (control – untreated) samples by 8.36%. Based on the resistance to termite classes, the untreated samples fell into class IV, in which the range of weight loss was 8.3% to 28.10%. Next, the treated samples of groups B, C, E, F, H, I, K, L, O, and P (Table 1) with weight losses ranging from 5.03% to 7.18% were classified into class III durability. The lowest weight loss due to drywood termite attack occurred in the group of treated samples J, N, M, D, and G, by 3.04%, 3.55%, 3.84%,

3.89%, and 4.27%, respectively, which classified these samples as resistance class II. The higher the weight loss gained due to drywood termite attack, the lower the wood durability. Based on the above results, Jabon wood

resistance against drywood termites can be improved from class IV to class III and II through impregnation and compression treatments.

Table 6. Weight loss, resistance, and mortality of drywood termites (*Cryptotermes cyanocephalus* Light) against Jabon wood.

Treatment	Weight Loss (%)	Resistance Class	Mortality (%)		Degree of Attack (%)
	X ± Sd*		%	X ± Sd*	
A (Control)	8.36 ± 1.51 a	IV	41.5	40.10 ± 1.74 e	25
B	5.90 ± 1.30 bc	III	44.5	41.82 ± 3.95 de	22.5
C	5.26 ± 1.07 bcde	III	46.5	42.99 ± 2.55 cde	20
D	3.99 ± 2.23 cde	II	59.0	51.07 ± 13.71 ab	15
E	6.98 ± 1.11 ab	III	50.0	45.00 ± 2.05 bcde	17.5
F	5.85 ± 1.40 bcd	III	51.5	45.86 ± 1.10 abcde	16.25
G	4.27 ± 1.41 cde	II	52.0	46.15 ± 0.94 abcde	13.75
H	5.87 ± 3.19 bc	III	52.5	46.44 ± 3.03 abcde	17
I	5.03 ± 0.08 bcde	III	50.5	45.29 ± 1.96 bcde	15.25
J	3.04 ± 0.66 e	II	57.0	49.05 ± 3.49 abc	13.75
K	5.35 ± 1.32 bcde	III	51.5	45.86 ± 1.10 abcde	14.25
L	5.16 ± 0.49 bcde	III	62.0	52.01 ± 4.98 ab	14.25
M	3.85 ± 0.49 cde	II	60.0	50.78 ± 1.91 ab	11.25
N	3.55 ± 0.20 de	II	63.0	52.57 ± 2.87 a	11.25
O	7.18 ± 1.52 ab	III	57.0	47.59 ± 1.72 abcd	21.25
P	6.87 ± 0.81 ab	III	51.5	45.86 ± 1.10 abcde	18.25

Remarks: A through P refers to Table 1; (*) The mean value followed by the same letter means no significant difference (p = 0.05) was determined based on Duncan post hoc test; Sd = Standard deviation.

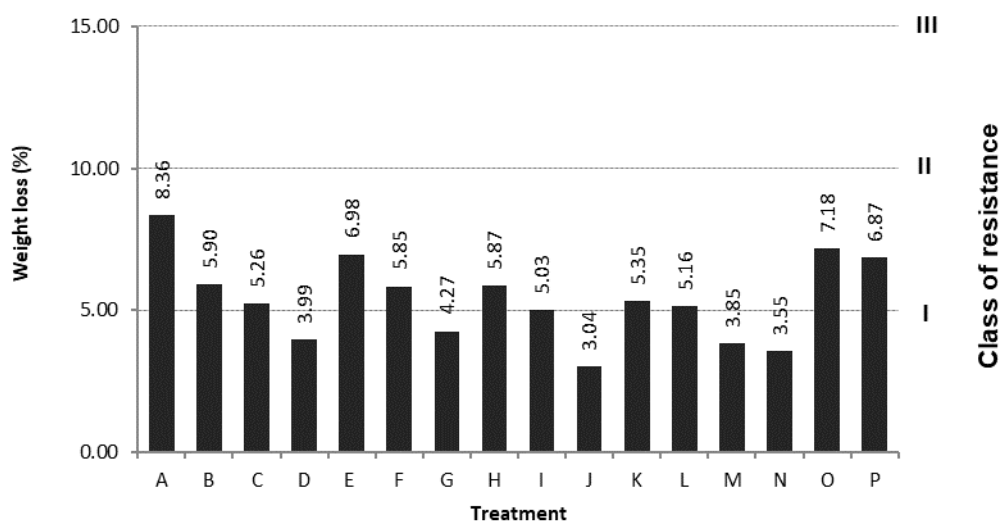


Figure 5. Weight loss of treated and untreated Jabon wood due to drywood termite attack.

The best treatment from the five treatments that resulted in class II can be determined by comparing the weight loss of these treatments with Duncan's multiple range test (Table 5). The table showed that the lowest weight loss belongs to treatment J (impregnated three times using PME33). However, Duncan's post hoc test revealed that at p = 0.05 the treatment did not make a significant difference in weight loss with the treatments of D, G, M, and N. As a result, these treatments are in the same class of resistance against drywood termites.

To choose the best treatment, the cost, safety factors, and technical aspects should be considered. However, based on the value of weight loss, treatment J resulted in the lowest weight loss. As mentioned above, this treatment did not show a significant difference with treatment D. Therefore, treatment D was considered the most efficient in preventing Jabon wood from drywood termite attack because of its cost (as there is no cost for other materials to make the polymer), its simplicity (as there is just the need for Merbau extractives for the treatment) and the safeness (it is the only natural compound required for the treatment).

This treatment did not need the polymerization process for Merbau extractives. Meanwhile, treatment G required polymerization to make polymerized Merbau extractives (PME) and the triple impregnation process. This can be an expensive and time consuming process. The samples of group M and N were made by impregnation with Merbau extractive and followed by hot compression by a 33% and 50% compression ratio, respectively. Both treatments were safe and an environmentally friendly, but required high energy for hot compression.

In terms of drywood termite mortality, the highest mortality of the drywood termites occurred on the treated wood of N (63%), L (62%), and M (60%) (Table 5). In contrast, the lowest mortality occurred on samples A, B, and C by 41.5%, 44.5%, and 46.5%, respectively. Based on the results, stronger treatments led to higher mortality. This means that the treatment of jabon wood increased its resistance against drywood termite attack.

This result is in line with the general theory of the role of extractive substances on the durability of wood, according to which extractives can protect wood against some biological damage or insect attack (Hillis 1987; Sjöström 1993; Shmulsky and Jones 2011). Syofuna *et al.* (2012) treated the wood of two susceptible species against termites and fungal decay, *Pinus caribaea* and *Antiaris toxicaria* using extractives obtained from *Milicia excelsa*, *Albizia coriaria*, and *Markhamia lutea*. The authors concluded that the extractives contributed greatly to the protection of less durable wood species against termite attack. In this study, Merbau extractives also distinctly improved the resistance of jabon wood from class IV (non-resistant) to class II (resistant).

Conclusions

Based on the testing of treated and untreated jabon wood against subterranean and drywood termites, this study showed that untreated samples of jabon belong to class V (susceptible) against subterranean termite and class IV (non-resistant) against drywood termite (BSN 2014).

The impregnation treatments and hot compression improved the resistance of jabon wood against subterranean and drywood termite attack. Impregnation using only Merbau extractives improved the resistance of jabon wood against subterranean termite from class V to class IV and against drywood termite from class IV to classes III and II.

Resistance class III against subterranean termites originated from the treatments of E, F, H, I, J, N, O, and P. Meanwhile, the same class of resistance against drywood termite attack was achieved by the treatments of B, C, E, F, H, I, K, L, O, and P.

Treatments G, K, L, and M for exposure to subterranean termites and treatments D, G, J, M, and N for exposure to drywood termites resulted in the resistance class of II. The treatment of J and D are recommended to

prevent jabon wood against subterranean and drywood termite attacks, respectively.

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Example of Table and Figure

Table 1. Effects of temperature on *in vitro* growth of seedlings.

Temp. (°C)	Shoot length (mm)	Number of leaf	Fresh weight (g)
25	59.2 ± 10.6 ^c	4.5 ± 0.8 ^a	0.29 ± 0.13 ^a
27	88.5 ± 9.3 ^a	4.8 ± 0.9 ^a	0.40 ± 0.12 ^a
29	75.0 ± 11.1 ^b	3.8 ± 0.6 ^a	0.30 ± 0.07 ^a

Note: Values (average ± standard deviation) with different letters are statistically significant according to Tukey's multiple comparison test. Data were recorded after 4 weeks of culture. MS medium was used as a basal medium without any PGRs. Number of sample = 10.

Source: Chujo *et al.* 2010.

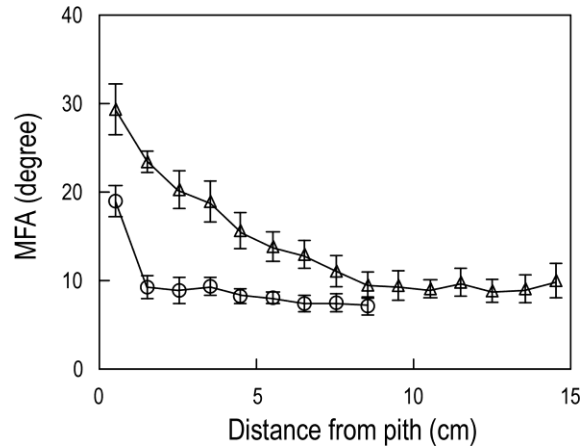


Figure 3. Radial variation of microfibril angle of the S2 layer in tracheid. Open circle, *Agathis* sp.; open triangle, *Pinus insularis*; Bars indicate the standard deviation. (Source: Ishiguri *et al.* 2010)

